GROWTH AND ONTOGENY OF SEA TURTLES USING SKELETOCHRONOLOGY: METHODS, VALIDATION AND APPLICATION TO CONSERVATION

by

Melissa Louise Snover

Duke University Marine Laboratory
Graduate Program in Ecology
Duke University

Date:_____

Approved:

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Dr. Larry B. Crowder, Supervisor

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Dissertation submitted in partial fulfillment of the requirement for the degree of
Doctor of Philosophy in the Graduate Program in Ecology
of the Graduate School of Duke University
2002

ABSTRACT

(Ecology)

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ABSTRACT

There are limits to the size of an individual that can be supported by a particular habitat. Organisms that span a large size range during their ontogeny often need to alter their life style in order to maintain optimal growth rates and minimize predation. Understanding the timing of ontogenetic habitat shifts is fundamental to the study of a species' life history and population dynamics. This information is especially critical to the conservation and management of threatened and endangered species, such as the loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidechelys kempi*) sea turtle. This dissertation investigates the application of skeletal growth marks (skeletochronology) to age estimation and growth rate analysis for loggerhead and Kemp's ridley sea turtles and the application of this technique in combination with stable isotope analyses of bone to identify ontogenetic shifts.

I validated the annual deposition of growth marks in known-age loggerhead and Kemp's ridleys. The timing of the formation of the lines of arrested growth in Kemp's occurred in the spring for animals that strand dead along the middle and south U.S. Atlantic coast. For both Kemp's and loggerheads, I found a proportional allometry between bone growth (humeri dimensions) and somatic growth (straight carapace length), indicating that size-at-age and growth rates can be estimated from dimensions of early growth marks. I analyzed stable isotope ratios within the bone tissue to track diet shifts that occur in conjunction with ontogenetic habitat shifts. For loggerheads, the transition to benthic habitats was accompanied with an increase in growth rates. In Kemp's, I found evidence of a potential secondary ontogenetic shift from analyses of the growth marks that resulted in increased growth rates. The duration of the pelagic stage in loggerheads was found to be

much longer than has previously been thought although the estimated age to maturity was still consistent with current estimates due to the indication of higher growth rates in the benthic stage. The results highlighted the use of skeletochronology as a valuable tool for the rapid assessment of growth rates in species where similar data can only be painstakingly gathered over long time periods.

ACKNOWLEDGEMENTS

This research was completed under and complied with the provisions of Sea Turtle Research Permit number TE-676379-2 issued by the U.S. Fish and Wildlife Service. The collection and processing of so many sea turtle bones would not have been possible to do alone. Most of the humeri were received through the cooperation of the Sea Turtle Stranding and Salvage Network, with special thanks to Ruth Beottcher and Wendy Teas. I thank all of the beach volunteers, park service personnel and stranding coordinators for getting flippers collected and shipped to me. In addition, humeri were received from Dr. Frank Swartz at the University of North Carolina – Chapel Hill Institute of Marine Science, Ben Higgins at the National Marine Fisheries Service – Galveston Lab, the Virginia Marine Science Museum Stranding Program, the Maryland Department of Natural Resources, and the Massachusetts Audubon Society in Wellfleet. In the lab I had help from numerous folks with 'flensing' sea turtle bones, which is a nice euphemism for what was actually involved, and I am indebted to them. I owe a special thanks to some very patient people who have assisted me over the years with sample preparation, Annie Gorgone, Bonnie Brown and Jerald Weaver.

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CHAPTER 1

INTRODUCTION: SEA TURTLE GROWTH AND LIFE HISTORY

OBJECTIVES

The objectives of this dissertation were:

1) to determine if the humerus bone in sea turtles contains annual growth marks for age estimation,

2) to determine if there is a constant proportional allometry between humerus dimension and body size to allow for estimation of somatic growth rates from growth mark dimensions,

3) to determine if the stable isotope ratios of bone tissue change in a manner consistent with prey sources to detect diet shifts,

4) to apply objectives 1-3 to the humeri of loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempi*) sea turtles for the purpose of assessing growth rates and the timing of ontogenetic shifts,

5) and to evaluate the implications of the timing of ontogenetic habitat shifts to conservation and our understanding of sea turtle life history .

BACKGROUND

Specific life histories of individual sea turtle species vary but the common denominator in all of them is that sea turtles are long-lived, slow-growing species that use multiple habitats over their course of development. Numerous authors have recently highlighted the management and conservation issues that are critical to maintaining species with this type of life history (Congdon et al. 1993, Heppell 1998, Crouse 1999, Heppell et al.

1999, Musick 1999). All of these authors highlight the need for high survival rates in the large juvenile, sub-adult and adult stages and/or the unlikelyhood that species with this life history are capable of withstanding sustained harvests in these stages, especially if the populations are already at reduced levels. To better understand the population dynamics of these long-lived species, we need a more thorough understanding of their life histories.

Life Stages and Ontogenetic Shifts

Organisms that span a large size range during their ontogeny often need to alter their life style in order to maintain optimal growth rates. Hence, the life cycles of many species incorporate shifts in habitat utilization, possibly to maximize growth rates while minimizing mortality risks (Werner and Gilliam 1984, Werner 1988). The timing of ontogenetic habitat shifts are critical parameters of a species life history, determining stage durations and ultimately time to reproductive maturation. Sea turtles species share a common life cycle composed of a series of ontogenetic stages. We know of the major ontogenetic shifts that are typified by discrete changes in habitat use, but there may be additional ontogenetic shifts in resource utilization of which we are not yet aware but may have important ramifications for management.

The general life cycle of a sea turtles begins when an adult female digs a nest cavity, usually on sandy, ocean-facing beaches and, depending on the species, deposits anywhere from 50 to 130 eggs per nest (Van Buskirk and Crowder 1994). Following incubation, hatchlings emerge from the nests, crawl down the beach to the water, and swim out to the open ocean (Lohman et al. 1997). Young juveniles remain pelagic for a period of time, the length of which varies by species and, potentially, by geographic location within species (Musick and Limpus 1997). Following the pelagic stage, juveniles of most species recruit to

nearshore habitats and switch to feeding on benthic organisms. For juveniles found in temperate regions, there are usually migrations between summer and winter habitats, while migrations are not as extensive for more tropical species (Musick and Limpus 1997).

A dramatic shift in habitat and diet occurs at least once in the life cycle of most juvenile sea turtles. This happens when juvenile sea turtles switch from a pelagic habitat and epipelagic feeding to a nearshore habitat and benthic feeding. Most of the species of sea turtles appear to spend little time as pelagic juveniles as they are seen in coastal habitat at small sizes (Marquez 1994, Chaloupka and Limpus 1997, Bjorndal et al. 2000a). Of the hard-shelled sea turtles, the loggerhead is an exception to this as they do not recruit to near shore habitats until they are 40-50 cm SCL for the SE USA population or greater than 70 cm SCL for Australia (Limpus et al. 1994, Bjorndal et al. 2000b).

For loggerheads nesting in the southeast United States, hatchlings and post-hatchlings are caught up in the Gulf Stream and eventually become entrained in the North Atlantic gyre (Carr 1987, Hays and Marsh 1997, Lohmann et al. 2001). Now juveniles, they remain pelagic, completing a full transatlantic migration before returning to the western North Atlantic (Carr 1987, Bolten et al. 1998). After juvenile loggerheads return to the western North Atlantic, they enter a benthic life stage, inhabiting coastal waters of the mid- and southeast United States for the duration of their life (Carr 1987). Settlement to the benthic habitats occurs between 40-60 cm SCL (straight carapace length) (Carr 1987, Bjorndal et al. 2000b). From an analysis of mark-recapture data of nesting females, mean size of nesting females at their first tagging event is estimated at 90 cm SCL (NMFS-SEFSC 2001).

Nesting for the Kemp's ridley sea turtle (*Lepidochelys kempi*) only occurs in the western Gulf of Mexico, primarily at Rancho Nuevo in Tamaulipas, Mexico. Hatchlings from these beaches are probably initially entrained in the Mexican Current, then into the current system in the northern Gulf of Mexico traveling eastward (Collard 1990). During their pelagic stage, they likely either remain in the Gulf of Mexico or enter the Loop current, then the Florida Current, and eventually the Gulf Stream moving as far north as Cape Cod, MA (Lazell 1980, Collard and Ogren 1990). Kemp's ridleys recruit to the benthic habitats along the Atlantic and Gulf of Mexico coasts between 20-25 cm SCL (Marquez 1994). Kemp's ridleys have been observed nesting between 58.5 and 72.5cm SCL (Marquez 1994).

The determination of age, growth rates, and stage duration in sea turtle species have relied on mark-recapture data and skeletochronology. The predominant method used to infer age-based growth rates and stage duration in sea turtles has been the comparison of growth in carapace length between captures/recaptures of tagged individuals. This information is used in the interval forms of the von Bertalanffy and/or logistic growth equations to produce a size-at-age growth curve (Fabens 1965, for example see Frazer 1987). Due to the inaccessibility of all life stages, these curves are often prepared from data that only spans a portion of the life stages. Therefore, these data can only be used to estimate the length of time it takes an animal to growth through the size classes found in the studied life stages (Bjorndal and Bolten 1988, Bjorndal et al. 1995, NMFS-SEFSC 2001, Braun-McNeill et al. in prep).

Skeletochronology uses growth marks found in bone tissue to estimate age. Numerous studies have applied this technique to sea turtles (Zug et al. 1986, Klinger and Musick 1992, Zug et al. 1995, Zug and Parham 1996, Parham and Zug 1997, Zug et al.

1997, Bjorndal et al. 1998, Zug and Glor 1998, Cole et al. 2001, Zug et al. 2002). Klinger and Musick (1992) and Cole et al. (2001) present evidence of the annual nature of the growth marks for loggerhead and Kemp's ridley sea turtles, however the technique has not been validated in other species.

Impacts of ontogenetic habitat shifts

Each habitat that sea turtles use over their ontongeny has different environmental parameters such as food availability and temperature that will influence growth rates. Little information is available on sea turtle growth rates in the pelagic (although see Zug et al. 1995 and Bjorndal et al. 2000b). Chaloupka and Limpus (1997) and Limpus and Chaloupka (1997) noted increasing growth rates after settlement in the hawksbill and green sea turtles. Growth rates increased up until 50-60 cm CCL for the hawksbill and 60-63 cm CCL for the green. After these peaks, growth rates declined monotonically to adulthood in both species. Of course it cannot be known if the increasing growth rates post-settlement were continuing from the pelagic stages or if they were surges in growth after settlement. However, the results of these studies on sea turtle growth highlight the likelihood that sea turtle growth rates are compartmentalized and that shifts may occur in conjunction with ontogenetic habitat shifts.

Recent studies have highlighted the possibility of shifts in growth rates that do not occur in conjunction with previously defined ontogenetic habitat shifts. Chaloupka (1998) analyzed age data from a study by Zug et al. (1995) and found evidence of separate growth compartments, or polyphasic growth within the pelagic stage of loggerheads in the Pacific Ocean. Similarly, with the Kemp's ridley, Chaloupka and Zug (1997) found evidence of polyphasic growth, with growth rates increasing at approximately 45 cm SCL. This

observation is supported by Schmid (1998) who found that, though not significant, average growth rates in the 40-50 cm SCL size class were higher than in 30-40 and 50-60 cm SCL size classes. These growth shifts do not relate to major ontogenetic habitat shifts but may be indicative of additional ontogenetic shifts, whether they be diet, habitat, or physiological in nature, that result in changes in growth rate.

In addition to the potential for changes in growth rates, different habitats present different hazards that are sources of mortality. Though different predation sources will exist in different habitats, for larger juveniles and adults the primary hazards are anthropogenic sources of mortality such as fishing gear and pollution (NRC 1990, TEWG 2000).

The degree of overlap between ontogenetic stages influences population stability, for example, by influencing how much the density of one size class effects the density of another size class through intraspecific competition. Variability of behavioral shifts in habitat use increases the range of population structures and dynamics that may be observed due to the resulting variability in size-specific mortality and growth rates (Gilliam and Fraser 1988).

METHODS

Age Estimation Techniques

In a review of age estimation techniques for amphibians and reptiles, Halliday and Verrell (1988) considered first mark-recapture and second skeletochronology as the most reliable methods. The multiple habitats, extensive migrations and long life-spans of sea turtles make them less than ideal candidates for mark-recapture studies. Hence,

skeletochronology, the use of periodic growth marks in bone to estimate age, is an important technique to develop and validate for these species.

The basic tenet of skeletochronology is that bone growth is cyclic and there is an annual periodicity in which bone formation ceases or slows before new, relatively rapid bone formation resumes (Simmons 1992, Castanet et al. 1993, Klevezal 1996). This interruption of bone formation is evidenced within the primary periosteal compacta by histological features, which take two forms in decalcified thin-sections stained with hematoxylin. The most common form is a thin line that appears darker than the surrounding tissue, termed line of arrested growth (LAG) (Castanet et al. 1977). The second, less common form is a broader and less distinct line that also stains darker, referred to as an annulus (Castanet et al. 1977). Alternating with LAGs or annuli are broad zones that stain homogeneously light and represent areas of active bone formation. Together, a broad zone followed by either a LAG or an annulus comprises a skeletal growth mark (GM) (Castanet et al. 1993). To apply skeletochronology to a species,

To apply skeletochronology to a species, the annual periodicity of the GMs must be validated. Klinger and Musick (1992, 1995) and Coles et al. (2001) have validated annual deposition of LAGs for the loggerhead sea turtle, *Caretta caretta,* with a tetracycline injection mark-recapture study on juveniles and adults in the Chesapeake Bay. Other skeletochronological studies of sea turtles have either not attempted validation of the annual deposition of the growth marks (Zug et al. 1986, Zug et al. 1995, Zug and Parham 1996, Parham and Zug 1997, Zug et al. 1997, Zug and Glor 1998, Zug et al. 2002) or have attempted and have not been able to identify the marks (Bjorndal et al. 1998). Smirina (1994) and Castanet (1994) review age estimation in amphibians and reptiles, respectively.

In their review of skeletochronological studies, those that validated deposition of the growth marks using known-age animals, mark-recapture, or tetracycline injection found the periodicity was annual.

Castanet et al. (1977) introduced the term 'line of arrested growth', or LAG, to identify the thin dark lines. The rational of this term is that this line in poikilotherms is a result of low metabolism and no growth associated with seasonal climate changes. In bone morphology, these structures are in the general class of cement or cementing lines and are common throughout all vertebrate bones. Resorption cement lines are found around Haversian canal systems, differentiating them from cortical bone, and in the lamellar deposition of secondary endosteal bone. Resting cement lines (the class that LAGs belong to) are found in the layering pattern of periosteal deposition of new cortical bone (Enlow 1969, Francillon-Vieillot et al. 1990). Resting cement lines also occur with annual periodicity in the cortical bone of mammals that do not generally lower metabolism or cease growing in cold seasons (Klevezal 1996). The reason for this is not understood though it may be associated with a spring surge in growth rates (Schauble 1972, Simmons 1992). Castanet et al. (1993) extended the terminology of LAGs to both poikilotherms and endotherms as a general description of a resting cement line marking periodicity in growth. They indicate that the formation of these lines is likely endogenous but potentially synchronized to environmental conditions.

In general, long bones such as the humerus, femur or phalanges, are the preferred skeletal element for application of the technique (Castanet et al. 1993). For the purpose of non-destructive sampling, other types of bone have been used such as osteoderms and caudal vertebrae (Tucker 1997, Waye and Gregory 1998).

Detection of Ontogenetic Habitat Shifts from Bone

The presence of growth marks in bone tissue provides chronological benchmarks that can be used to assess growth and diet. The spacing of the LAGs provides information on proportional growth (see below). In addition, unresorbed bone tissue provides a dietary record, through stable isotope ratios, of the food being consumed at the time the bone was deposited. As noted previously, a major ontogenetic shift for sea turtles is the shift from the pelagic habitats to the benthic habitats. The bone tissue of juvenile benthic sea turtles, then, should contain a record of this habitat shift.

A series of papers on the Australian freshwater crocodile, *Crocodylus johnstoni*, highlighted an ontogenetic habitat and diet shift that seems to correlate with separate growth compartments indicative of polyphasic growth rates (Tucker et al 1996, Tucker 1997, Tucker et al 1997). For sea turtles, researchers have found evidence of polyphasic growth rates in Kemp's ridley, green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), and Pacific loggerhead (Chaloupka and Limpus 1997, Chaloupka and Zug 1997, Limpus and Chaloupka 1997, Chaloupka 1998) by analyzing size-at-age from mark-recapture or skeletochronology data. For green and hawksbill, Chaloupka and Limpus (1997) and Limpus and Chaloupka (1997) suggest that somatic growth rates for these animals consist of the juvenile pelagic stage and the juvenile benthic stage with differential growth rates in these two stages. Such a shift in growth rates should be evident from the spacing of the growth marks in cross-sections of humeri of benthic juveniles.

Stable isotopes from bone and tooth collagen have been used to track migration patterns and trophic relationships in marine systems (Hobson and Welch 1993, Godley et al. 1998, Burton and Koch 1999, Walker and Macko 1999, Walker et al. 1999). The isotopic

composition of a consumer is consistent with, or deviates by, a constant amount from its food source. Specifically, the ratio of ^{15}N to ^{14}N ($\delta^{15}N$) undergoes an approximately 3 ‰ increase at each trophic level in the food web. For carbon, the ratio of ^{13}C to ^{12}C ($\delta^{13}C$), the value is about 1 ‰ (Michener and Schell 1994). The photosynthetic organisms forming the base of the food web also have distinctive $\delta^{13}C$ values. *Spartina* grasses have $\delta^{13}C$ values of around -12 to -13 ‰ while pelagic plankton values are around -21 ‰ (Peterson and Howarth 1985). By analyzing $\delta^{15}N$ and $\delta^{13}C$ values in different regions of humeri cross-sections where growth rate shifts seem evident from the growth marks, a shift in diet can be detected. The diet shift can be related to habitat use by analyzing the stable isotopic composition of representative prey items from each habitat.

Estimation of growth rates from bone

Growth marks found in bones can be used to back-calculate the size of the animal at the time the mark was deposited by comparing the dimensions of the growth mark to a measure of individual body size. This method has been used extensively in fish using scales, otoliths or vertebrae (Francis 1990). Klevezal (1996) has found that growth patterns of the rodent *Marmota bailbacina* is synchronous with growth layer formation in bone and that individual growth patterns can be determined from skeletal growth marks in mammals.

The first step is to establish that a significant correlation exists between a bone dimension, such as the diameter of the diaphyses, and animal size. The diameters of the LAGs within the diaphyses of the bone, then, can be used to back-calculate the size of the animal using the regression model. Caetano and Leclair (1996) applied this method to red-spotted newts (*Notophthalmus viridescens*) using the circumference of the LAGs in cross-

sections of the humerus. More recently, Bjorndal et al. (in review) it has applied this technique to analyze growth rates in pelagic stage loggerheads.

This method can be validated to a certain extent by comparing the back-calculated size-at-ages to those directly taken from approximated age vs. body size. Also, previously captured and measured individuals recovered as dead strandings can be used to assess the accuracy of estimating growth rates from skeletal growth marks. Information from this analysis provides rapid and numerous data on actual size-specific growth rates currently only painstakingly acquired from mark-recapture studies. It can also provide information on growth rates for earlier life stages that are may not otherwise be available.

Application of stage durations to population dynamics

Matrix projection models have been developed and refined for loggerhead and Kemp's ridley sea turtles (Crouse et al. 1987, Crowder et al. 1994, Heppell et al. 1996a, Heppell et al. 1996b, Turtle Expert Working Group 2000, Heppell et al. 2002a). These models have been invaluable in guiding management decisions for these species. Elasticity analyses of these models have demonstrated the importance of using TEDs to protect the large juvenile and sub-adult stages as a means of increasing population growth rate (Crowder et al. 1994, Heppell et al. 1996a). A better understanding of stage durations will allow for an updated analysis of the models and the resulting stage elasticities, which are very sensitive to stage length.

SUMMARY

This dissertation investigates the application of skeletal growth marks to age estimation and growth rate analysis for loggerhead and Kemp's ridley sea turtles and the

application of this technique alone and in combination with stable isotope analyses to identify ontogenetic shifts. In chapter two I review validated skeletochronological studies of amphibians and reptiles. I include analyses of known-age sea turtle specimens that I have received and my findings on the timing of the deposition of LAGs in loggerhead and Kemp's ridley sea turtles. I also detail the methodology for bone selection, preparation, and interpretation of growth marks for age estimation in sea turtle bones. In chapter three I establish a settlement line in loggerhead humeri that marks the shift from pelagic to benthic habitats. I use changes in growth rates evidenced from growth marks and changes in stable isotope ratios of the bone tissue to support the hypothesis of the settlement line. Once the timing of the shift is established, I use individual growth trajectories to estimate the duration of the pelagic stage. In chapter four I apply the same techniques to Kemp's ridley sea turtles. As Kemp's have a much shorter pelagic phase than loggerheads, the identification of when they settle out of the pelagia is made by examining characteristic growth marks in small animals and verifying the habitat shift with stable isotopes. I estimate age from direct counts of growth marks up to about age seven, then use growth rates in animals of all sizes to complete the growth curve and approximate average age to maturation. I analyze variance in growth rates and size-at-age for the detection of shifts in growth rates indications polyphasic growth rates. In chapter 5, I complete the evaluation of the loggerhead life cycle by estimating the duration of the benthic juvenile stage and combining information from Chapter 3 to estimate age at maturation. This information is used to update estimates of survival rates and stage durations in the current loggerhead matrix model (Heppell et al. 2002). The model is then used to investigate the impact of increased

pelagic stage mortality from pelagic fisheries and decreased benthic mortality from the use of turtle excluder devices in the shrimp trawl fishery.

CHAPTER 2

VALIDATION OF ANNUAL GROWTH MARK DEPOSITION AND INTERPRETATION IN LOGGERHEAD (*CARETTA CARETTA*) AND KEMP'S RIDLEY (*LEPIDOCHELYS KEMPI*) SEA TURTLES

INTRODUCTION

The basic tenet of skeletochronology is that bone growth is cyclic and there is an annual periodicity in which bone formation ceases or slows before new, relatively rapid bone formation resumes (Simmons 1992, Castanet et al. 1993, Klevezal 1996). This interruption of bone formation is evidenced within the primary periosteal compacta by histological features, which take two forms in decalcified thin-sections stained with hematoxylin. The most common form is a thin line that appears darker than the surrounding tissue, termed line of arrested growth (LAG) (Castanet et al. 1977). The second, less common form is a broader and less distinct line that also stains darker, referred to as an annulus (Castanet et al. 1977). Alternating with LAGs or annuli are broad zones that stain homogeneously light and represent areas of active bone formation. Together, a broad zone followed by either a LAG or an annulus comprises a skeletal growth mark (GM) (Castanet et al. 1993). To apply skeletochronology to a species, the annual periodicity of the GM's must be validated.

Three common methods can be employed to validate annual deposition of growth marks; 1) the study of known-age animals, 2) mark-recapture studies, and 3) mark-recapture studies that incorporate fluorescent marking (Castanet 1994). These studies often require the sacrifice of specimens (Collins and Rodda 1994, Wake and Castanet 1995, Caetano and Leclair 1999, de Buffrenil and Castanet 2000). Alternatively, in some species, bones such as osteoderms, caudal vertebrae or distal phalanges can be removed without serious injury from

live specimens (Kusano et al. 1995, Fretey and Le Garff 1996, Friedl and Klump 1997, Tucker 1997, Waye and Gregory 1998, Driscoll 1999, Waye 1999, Trenham et al. 2000).

Skeletochronology has become a commonly used tool in studies of amphibian and, to a lesser extent, reptilian populations, with the annual nature of the growth marks often assumed, as evidenced by studies published since 1992 (Table 2.1). Only a quarter of these published studies document annual deposition. If one considers species for which a previous study has confirmed annual growth marks, then 40% of the studies published since 1992 have been applied to species for which annual GM's have been validated (Table 2.2). Still, the majority of studies utilizing skeletochronology are applying the technique as a tool that has not been validated for their species.

Validation studies are necessary not only to confirm the annual nature of the growth marks, but also to identify and interpret anomalous LAGs. Anomalies include double, splitting, and supplemental LAGs (Castanet et al. 1993). Double LAGs (two distinct LAGs spaced close together) are observed in reptiles (Chinsamy et al. 1995, El Mouden et al. 1997) and amphibians (Caetano and Castanet 1993, Caetano and Leclair 1996, Guarino et al. 1998). Generally, double LAGs are interpreted as one mark. Splitting LAGs (thick LAGs that split into two or more thinner LAGs) have been interpreted as both distinct annual lines (Guarino et al. 1995, 1998) and as one line

(Coles et al. 2001). Supplemental lines are thin lines similar to LAGs that appear within zones (Guarino et al. 1995, Tinslet and Tocque 1995, Caetano and Leclair 1996, El Mouden et al. 1997, Guarino et al. 1998, Lima et al. 2000 and Trenham et al. 2000). Supplemental lines are easily identified as they do not appear around the full circumference of cross-sections and have weak optical contrast. In an analysis of known-age California tiger

Table 2.1. Species and validation results from skeletochronological studies published since 1992. Numbers indicating validation methods are (1) known-age, (2) mark-release-recapture, (3) mark-release-recapture with florescent marking and 4) comparison of results from two or more methods of age estimation.

Group	Species	Validation	Evidence of Annual GM's?	Source
Reptilia				
Testudines	*Caretta caretta*	3	Yes	Coles *et al.* (2001)
	Caretta caretta	-	-	Parham and Zug (1997)
	Caretta caretta	-	-	Zug *et al.* (1995)
	Caretta caretta	3	Yes	Klinger and Musick (1992)
	Caretta caretta	-	-	Klinger and Musick (1995)
	Chelonia mydas	3	No	Bjorndal *et al.* (1998)
	Chelonia mydas	-	-	Zug and Glor (1998)
	Chelonia mydas	-	-	Zug et al. (2002)
	Lepidochelys kempi	-	-	Zug *et al.* (1997)
Squamata – Sauria	*Agama impalearis*	-	-	El Mouden *et al.* (1997)
	Angolosaurus skoogi	-	-	Chinsamy et al. (1995)
	Varanus niloticus	3	Yes	De Buffrenil and Castanet (2000)
	Varanus niloticus	-	-	De Buffrenil et al. (1999)
	Varanus niloticus	-	-	De Buffrenil et al. (1994)
Squamata - Serpentes	*Bioga irregularis*	3	No	Collins and Rodda (1994)
	Thamnophis elegans	1	Yes	Waye and Gregory (1998)
	Thamnophis elegans	2	Yes	Waye (1999)
Crocodylia	*Crocodylus johnstonii*	2	Yes	Tucker (1997)

Table 2.1, continued

Amphibia				
Urodela	*Ambystoma californiense*	2	Yes	Trenham *et al.* (2000)
	Ambystoma macrodactylum	-	-	Russell *et al.* (1996)
	Ambystoma maculatum	-	-	Flageole and Leclair (1992)
	Batrachoseps attenuatus	4	Yes	Wake and Castanet (1995)
	Chioglossa lusitanica	-	-	Lima et al. (2000)
	Cynops pyrrhogaster	-	-	Marunouchi et al. (2000)
	Desmognathus monticola	-	-	Castanet *et al.* (1996)
	Desmognathus quadramaculatus	-	-	Castanet *et al.* (1996)
	Desmognathus ochrophaeus	-	-	Castanet *et al.* (1996)
	Hynobius kimurae	-	-	Misawa and Matsui (1999)
	Notophthalmus viridescens	-	-	Caetano and Leclair (1996)
	Phaeognathus hubrichti	-	-	Parham *et al.* (1996)
	Salamandra salamandra	-	-	Alcobendas and Castanet (2000)
	Triturus alpestris	-	-	Miaud et al (2000)
	Triturus alpestris	-	-	Joly and Grolet (1996)
	Triturus boscai	1	Yes	Caetano and Leclair (1999)
	Triturus cristatus	-	-	Miaud et al. (1993)
	Triturus marmoratus pygmaeus	-	-	Diaz-Paniagua et al. (1996)
	Triturus marmoratus	-	-	Caetano and Castanet (1993)
Anurans	*Alytes cisternasii*	-	-	Marquez *et al.* (1997)
	Alytes obstetricans	-	-	Marquez *et al.* (1997)
	Bombina variegata pachypus	-	-	Guarino et al. (1995)
	Bufo Bufo	2	Yes	Fretey and Le Garff (1996)
	Bufo calamita	-	-	Denton and Beebee (1993)
	Bufo cognatus	-	-	Rogers and Harvey (1994)
	Bufo pardalis	-	-	Cherry et al. (1992)
	Bufo raddei	-	-	Kuzmin and Ischenko (1997)
	Bufo viridis	-	-	Castellano et al. (1999)
	Bufo woodhousii fowleri	-	-	Kellner and Green (1995)
	Discoglossus galganoi	-	-	Esteban *et al.* (1998)
	Geocrinia alba	2	Yes	Driscoll (1999)
	Geocrinia vitellina	2	Yes	Driscoll (1999)
	Hyla arborea	2	Yes	Friedl and Klump (1997)
	Hynobius kimurae	-	-	Misawa and Matsui (1999)

Table 2.1. continued

Anurans	*Mantidactylus microtympanum*	-	-	Guarino *et al.* (1998)
	Pelobates fuscus	-	-	Eggert and Guytant (1999)
	Rana cyanophlyctis	-	-	Pancharatna et al. (2000)
	Rana cyanophlyctis	-	-	Kulkarni and Pancharatna (1996)
	Rana dalmatina	-	-	Guarino et al. (1995)
	Rana italica	-	-	Guarino et al. (1995)
	Rana luteiventris	-	-	Reaser (2000)
	Rana perezi	-	-	Estaban et al. (1996)
	Rana saharica	-	-	Esteban et al. (1999)
	Rana sakuraii	4	Yes	Kusano et al. (1995)
	Rana septentrionalis	-	-	Leclair and Laurin (1996)
	Rana sylvatica	-	-	Leclair et al. (2000)
	Rana sylvatica	-	-	Sagor *et al.* (1998)
	Rana sylvatica	-	-	Bastien and Leclair (1992)
	Rana temporaria	-	-	Miaud *et al.* (1999)
	Rana temporaria	-	-	Ryser (1996)
	Rana temporaria	-	-	Augert and Joly (1993)
	Scaphiopus couchii	-	-	Tinsley and Tocque (1995)

Table 2.2. Species for which annual growth marks have been validated. Numbers indicating validation method are (1) known-age, (2) mark-release-recapture, (3) mark-release-recapture with flourescent marking, (4) comparing results from two or more methods of age estimation, and (5) known-age with flourescent marking.

Group	Species	Validation	Source
Reptilia			
Testudines	*Emys orbicularis*	3	Castanet (1985)
	Testudo hermanni	1	Castanet and Cheylan (1979)
	Testudo hermanni	3	Castanet (1985)
	Testudo graeca	1	Castanet and Cheylan (1979)
Squamata-Sauria	*Cophosaurus texanus*	2	Nouira et al. (1982)
	Lacerta lepida	1	Castanet (1985)
	Lacerta viridis	2	Saint Girons et al. (1989)
	Lacerta viridis	3	Castanet (1985)
	Lacerta vivipara	4	Pilorge and Castanet (1981)
Squamata-Serpentes	*Vipera aspis*	3	Castanet and Naulleau (1985)
Crocodylia	*Crocodylus niloticus*	5	Hutton (1986)
Amphibia			
Urodela	*Desmognathus ochrophaeus*	3	Kazmer (1986)
	Notophtalmus viridescens	3	Kazmer (1986)
	Triturus cristatus	3	Francillon (1979)
	Triturus marmoratus	2	Caetano (1988), ref in Castanet et al.
Anurans	*Bufo americanus*	2	Kalb and Zug (1990)
	Bufo bufo	2	Hemelaar and van Gelder (1980)
	Rana catasbeiana	1	Schroeder and Baskett (1968)
	Rana esculenta	3	Francillon and Castanet (1985)
	Rana temporaria	3	Smirina (1972)

salamanders, Trenham et al. (2000) confirm that supplemental lines are not annual marks.

In addition to anomalous LAGs, there are two other difficulties typical in skeletochronology studies; compression of LAGs at the periphery of the bone and resorption of the innermost LAGs. In older animals the growth marks are compressed at the outer periphery of the bone as a result of decreased growth. Francillon-Vieillot et al. (1990) term this phenomenon 'rapprochement' and it is considered a problem when the LAGs become too close together to differentiate, usually in the small phalangeal bones used in amphibian studies (Miaud et al. 1993, Miaud et al. 1999, Eggert and Guyetant 1999, Lima et al. 2000 and Leclair et al. 2000). The reduction in growth rates indicated by rapprochement is likely linked to the onset of sexual maturity (Senning 1940, Kleinenberg and Smirina 1969 and Gibbons and McCarthy 1983, El Mouden et al. 1997). However, rapprochement was not identified in all analyzed samples of breeding common frogs (Augert and Joly 1993).

In most of the studies, resorption of the innermost (earliest) growth marks is considered a minimal problem. Usually when resorption occurs, fewer than three LAGs are completely lost and these can be accounted for (Bastien and Leclair 1992, Kusano et al. 1995, Castanet et al 1996, Sagor et al. 1998). In a few studies resorption is a more serious problem. Parham et al. (1996) found complete resorption of all the interior growth marks in three of 11 samples of red hills salamanders. Growth mark counts in the osteoderms of known-history breeding female freshwater crocodiles are more than 10 years too low (Tucker 1997). The problem is also extreme in age-estimation studies of sea turtles (Klinger and Musick 1995, Zug et al. 1995, Zug et al. 1997, Parham and Zug 1997, Zug and Glor 1998, Zug et al. 2002). In each of these studies, the authors used unvalidated back-calculation techniques to

estimate the number of layers lost.

Back-calculation techniques in sea turtles rely on the concept that the spatial pattern of the LAGs is representative of the growth of the animal. To confirm this assumption, researchers must establish a correlation between bone dimensions and body size (Hutton 1986, Klinger and Musick 1992, Leclair and Laurin 1996).

Applying the above validation methods and interpretation of LAGs to the study of sea turtles is complicated as the multiple habitats, extensive migrations, and long lifespans of sea turtles make them less than ideal candidates for mark-recapture studies. Also, no bone can easily be sampled from live individuals without harm. Sea turtle species' status of threatened or endangered (2000 International Union for Conservation of Nature and Natural Resources (IUCN) Red List of threatened species, US Endangered Species Act) means removing animals from the population for aging is inconsistent with conservation efforts.

Despite the difficulties, numerous studies have applied skeletochronology to sea turtles (Zug et al. 1986, Klinger and Musick 1992, Klinger and Musick 1995, Zug et al. 1995, Zug and Parham 1996, Parham and Zug 1997, Zug et al. 1997, Bjorndal et al. 1998, Zug and Glor 1998, Coles et al. 2001, Zug et al. 2002). A tetracycline-injection study validated annual growth marks for juvenile loggerhead sea turtles, *Caretta caretta*, from the Chesapeake Bay (Klinger and Musick 1992). An adult loggerhead from that same study stranded dead 8.25 yrs after injection and provides evidence of annual deposition in adults (Coles et al. 2001). Two of the studies that attempted to validate skeletochronology in sea turtles use bone biopsy (core) samples as an alternative to sacrificing the animal upon recapture (Klinger and Musick 1992, Bjorndal et al. 1998). Bone cores are not ideal for

skeletochronology because accurate interpretation of LAGs requires following them around the entire circumference of cross-sections. Except for the single animal highlighted in Coles et al. (2001), other skeletochronological studies of sea turtles use samples from dead animals and did not validate that growth marks are annual (Zug et al. 1986, Zug et al. 1995, Zug and Parham 1996, Parham and Zug 1997, Zug et al. 1997, Zug and Glor 1998, Zug et al. 2002).

What is needed, then, for the appropriate application of skeletochronology to sea turtle species is validation of annual growth marks and a guide to their interpretation. As resorption is a problem in sea turtle bones, the validation of a proportional allometry between bone and somatic growth is necessary to aid back-calculation. In this chapter, I address each of these issues for Kemp's ridley (*Lepidochelys kempi*) and loggerhead (*Caretta caretta*) sea turtles.

MATERIALS AND METHODS

I obtained samples from two known-age loggerheads and nine known-age Kemp's ridleys. I also received samples from five loggerheads that had been captured, measured, tagged, released, and eventually recovered as dead strandings (Table 2.3). In addition, I collected samples from 266 wild loggerheads and 291 wild Kemp's ridleys.

Sample preparation

Zug et al. (1986) analyzed skeletal elements of the cranium and right forelimb of loggerhead sea turtles and determined that the humerus was most suited to skeletochronological studies. Therefore, I also used the humerus. Specimens arrived as either dried bones or whole flippers. For flippers, I dissected out the humerus, which was then flensed, boiled, and air-dried for at least two weeks.

Table 2.3. Species and history of known-age sea turtles analyzed in this study.

Sample ID	Species	**Captive History**	**Age (yrs.)**
LK-1	*Lepidochelys kempi*	Captive for first year then released	4.5
LK-2	*Lepidochelys kempi*	Captive for first year then released	6.5
LK-3	*Lepidochelys kempi*	Captive for first year then released	5.0
LK-4	*Lepidochelys kempi*	Tagged and released post-hatching	1.27
LK-5	*Lepidochelys kempi*	Tagged and released post-hatching	1.70
LK-6	*Lepidochelys kempi*	Tagged and released post-hatching	1.72
LK-7	*Lepidochelys kempi*	Tagged and released post-hatching	2.37
LK-8	*Lepidochelys kempi*	Tagged and released post-hatching	2.37
LK-9	*Lepidochelys kempi*	Tagged and released post-hatching	3.25
LK-10	*Lepidochelys kempi*	Tagged and released post-hatching	2.0
LK-11	*Lepidochelys kempi*	Tagged and released post-hatching	2.75
LK-12	*Lepidochelys kempi*	Tagged and released post-hatching	3.0
LK-13	*Lepidochelys kempi*	Tagged and released post-hatching	4.25
CC-1	*Caretta caretta*	Captive entire life	29.4
CC-2	*Caretta caretta*	Captive for first two years then released	8
CC-3	*Caretta caretta*	Tagged and released, at large for 2.5 years	-
CC-4	*Caretta caretta*	Tagged and released, at large for 3 years	-
CC-5	*Caretta caretta*	Tagged and released, at large for 2 years	-
CC-6	*Caretta caretta*	Tagged and released, at large for 3.5 years	-
CC-7	*Caretta caretta*	Tagged and released, at large for 2.5 years	-

I cross-sectioned each humerus at a site just distal to the deltopectoral crest. At this site, the ratio of cortical to cancellous bone is highest (Zug et al. 1986) and the region immediately distal to the insertion scar of the deltopectoral muscle on the ventral side of the bone maximizes that ratio (see Zug et al. 1986 for diagrams of the loggerhead sea turtle humerus). This site also provided a landmark allowing sectioning at equivalent sites on every humerus.

I removed 2-3mm thick sections at that site using a Buehler® isomet low speed saw. This section was fixed in 10% formalin then decalcified using a commercial decalcifying agent (RDO, Apex Engineering Products Corporation). Time to decalcification varied with the size of the bone and the strength of the solution, usually between 12 and 36 hours. Following decalcification, I made 25μm cross-sections using a freezing-stage microtome. Sections were stained in Erlich's hematoxylin diluted 1:1 with distilled water (Klevezal 1996) and mounted on slides in 100% glycerin.

Known-age sea turtles

I received the humeri from each of two captive, known-age loggerhead sea turtles after they died (Table 2.3). The first, CC-1, was held in an outdoor tank during the summer months and inside a greenhouse during the winter months (female noted in Swartz 1997). The second, CC-2, was raised in captivity for two years then released from Panama City, Florida, into the Gulf of Mexico. In addition, I received humeri from five loggerheads that were tagged as part of an ongoing mark-recapture program (Braun-McNeill et al. in prep). These animals were recovered at least two years after their initial tag date.

For Kemp's ridleys, I received humeri from 13 dead known-age animals (Table 2.3).

The headstart Kemp's ridleys were raised in captivity for one year then released as part of a binational program operated jointly by state and federal U.S. agencies and the Instituto Nacional de la Pesca (INP) of Mexico (Klima and McVey 1995). The Coded Wire Tagged (CWT) Kemp's ridleys were tagged and released as hatchlings. This tagging program is operated jointly by the U.S. National Marine Fisheries Service (NMFS) Galveston Laboratory and Instituto Nacional de la Pesca (INP) of Mexico as a means of gaining a better understanding of the early life history of the Kemp's ridley sea turtle (Caillouet et al. 1997).

Using the methods described previously, I prepared stained thin-sections from the humeri. Without prior knowledge of the animal's history, the number of visible LAGs was quantified for each bone and a minimum age estimated. My age estimates were then compared to the age information available for each animal. For the tagged wild loggerheads, diameters of the growth marks were measured from calibrated digital images. I converted millimeters of bone growth to centimeters of straight line carapace growth using the regression equation for humerus width on straight carapace length (see below, Table 2.5). I assumed that LAGs are deposited in the spring and estimated growth from the LAG that would be most representative of the time when the animal was marked. For example, for CC-3 I used the LAG deposited in spring 1996 to represent the 21 November 1995 initial tag date as the animals likely do not grow during the winter months. I calculated the amount of bone growth under three hypotheses, that LAGs are deposited once per year, twice per year and every other year.

Indirect validation of annual timing of LAG deposition

Peabody (1961) and Castanet et al. (1993) suggest a correlation between the width of

the last zone formed and date of death as an indirect means of assessing that the deposition of the LAGs occurs annually and at the same time of year for an individual population. I applied this method to 76 wild Kemp's ridleys for which humeri displayed between one and five LAGs. Each of these animals stranded dead along the Atlantic coast between Maryland and North Carolina, USA. Thin-sections were prepared of the humeri as described above. I quantified the width of the last zone formed by measuring the diameter of the whole section (DWS) and the diameter of the last competed LAG (DLL), between the lateral edges of the bone on an axis parallel to the dorsal edge. The amount of bone growth after the last LAG (DWS – DLL) was plotted against the Julian stranding date, making the assumption that stranding date approximated date of death. Least-squares linear regressions were fit to the data.

The relationship between LAG diameter and body size

In order to relate growth mark diameters to somatic growth rates, there must be a constant proportionality between bone growth and somatic growth (Chaloupka and Musick 1997). To address this, I took eight morphometric measurements of 240 wild loggerhead and 262 wild Kemp's ridley humeri using digital calipers or a tape measure when dimensions were beyond the range of the calipers; measurements included maximum length, longitudinal length, proximal width, distal width, deltopectoral crest width, lateral diameter at sectioning site, ventral to dorsal thickness at sectioning site and mass. I compared these measurements with the carapace length, measured as standard straight-line length (SCL), from the nuchal notch to the posterior end of the posterior marginal, using a least-squares linear regression. For mass, the data were natural log transformed to linearize the regression. To test the accuracy of estimating carapace length from the diameter of the sectioning site, I used the

resulting regression equation to estimate carapace length from 25 additional loggerhead humeri and 28 additional Kemp's humeri. Estimated carapace lengths were plotted against observed carapace lengths for each species and a least-squares linear regression was fit. A t-test was used to test the resulting slopes for significant difference from one.

RESULTS

Known-age sea turtles

Kemp's ridley (*Lepidochelys kempi*)

Three Kemp's captive for one year then released were recovered 4.5 to 6.5 years after hatching (Table 2.3). Sample LK-1 displayed four completed GM's (zones followed by LAGs) and one incomplete mark (zone without a LAG) (Fig. 2.1). Without prior knowledge of this animal's age, I estimated the age accurately at 4.5 years based on layer count and time of death. Sample LK-2 retained five completed and one incomplete GM's, but with a large area of resorption (Fig. 2.1). I aged this animal at a minimum of 5.5 years, the actual age being 6.5 years. Sample LK-3 had minimal resorption and five completed GM's (Fig. 2.1). Based on growth mark counts and death date, I estimated the age of this animal accurately at five years.

Ten of the Kemp's ridley samples were tagged and released after hatching, with no time spent in captivity (Table 2.3). Results from these ten recovered animals allowed us the opportunity to study and interpret the early growth mark patterns in non-captive animals. The first year mark for Kemp's appeared to be a poorly defined annulus, as evidenced by LK-4 (Fig.2.2). This mark appeared more or less distinctly in the other, >2-year-old CWT animals (Fig. 2.2 – 2.6), with the mark being very indistinct in LK-5 (Fig. 2.2). Additional

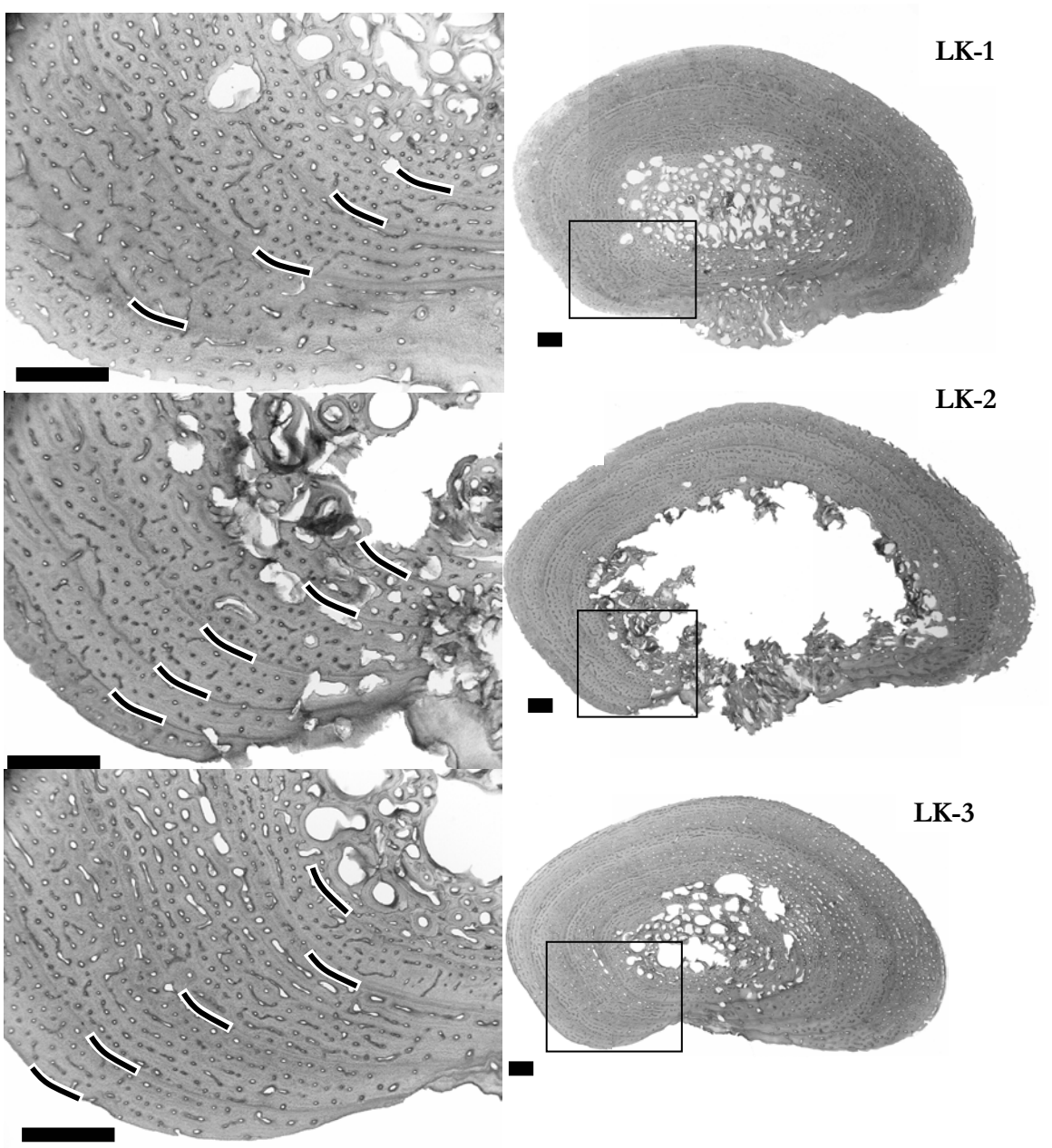


Figure 2.1. Images of humeri cross-sections of the three headstart Kemp's ridleys. Images to the right are low magnifications (7.5X) of the entire cross-section, regions of low magnification images outlined by rectangles are shown magnified to the left with curved black lines outlined in white highlighting the LAGs. Black bars represent 1mm in length; LK-1, 4.5 years old; LK-2, 6.5 years old; and LK-3, 5.0 years old.

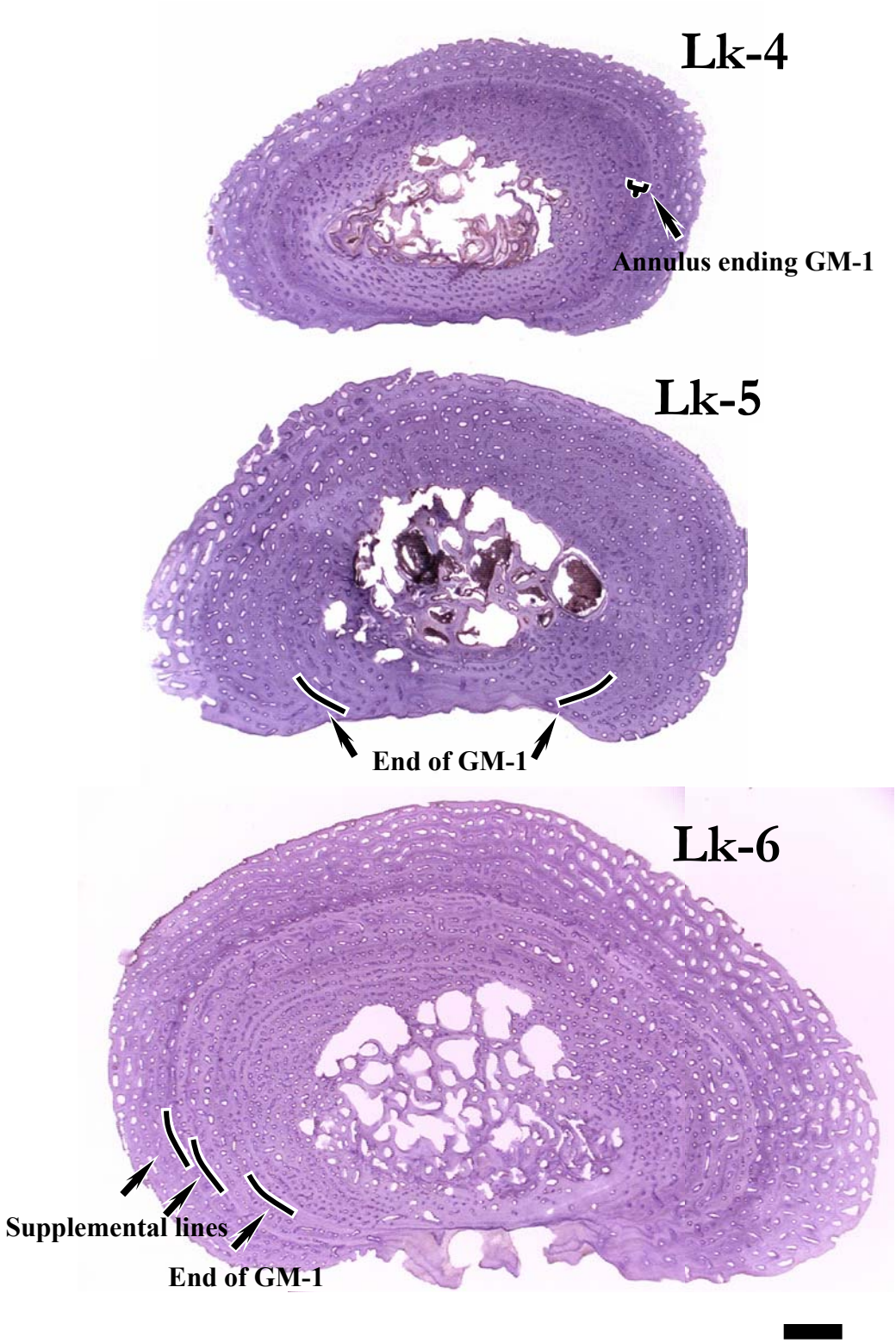


Figure 2.2. Images of humeri cross-sections of coded wire tagged Kemp's ridleys, GM-1 refers to growth mark one. Black bar represent 1mm in length; LK-4, 1.27 years old; LK-5, 2.37 years old; and LK-6, 2.37 years old.

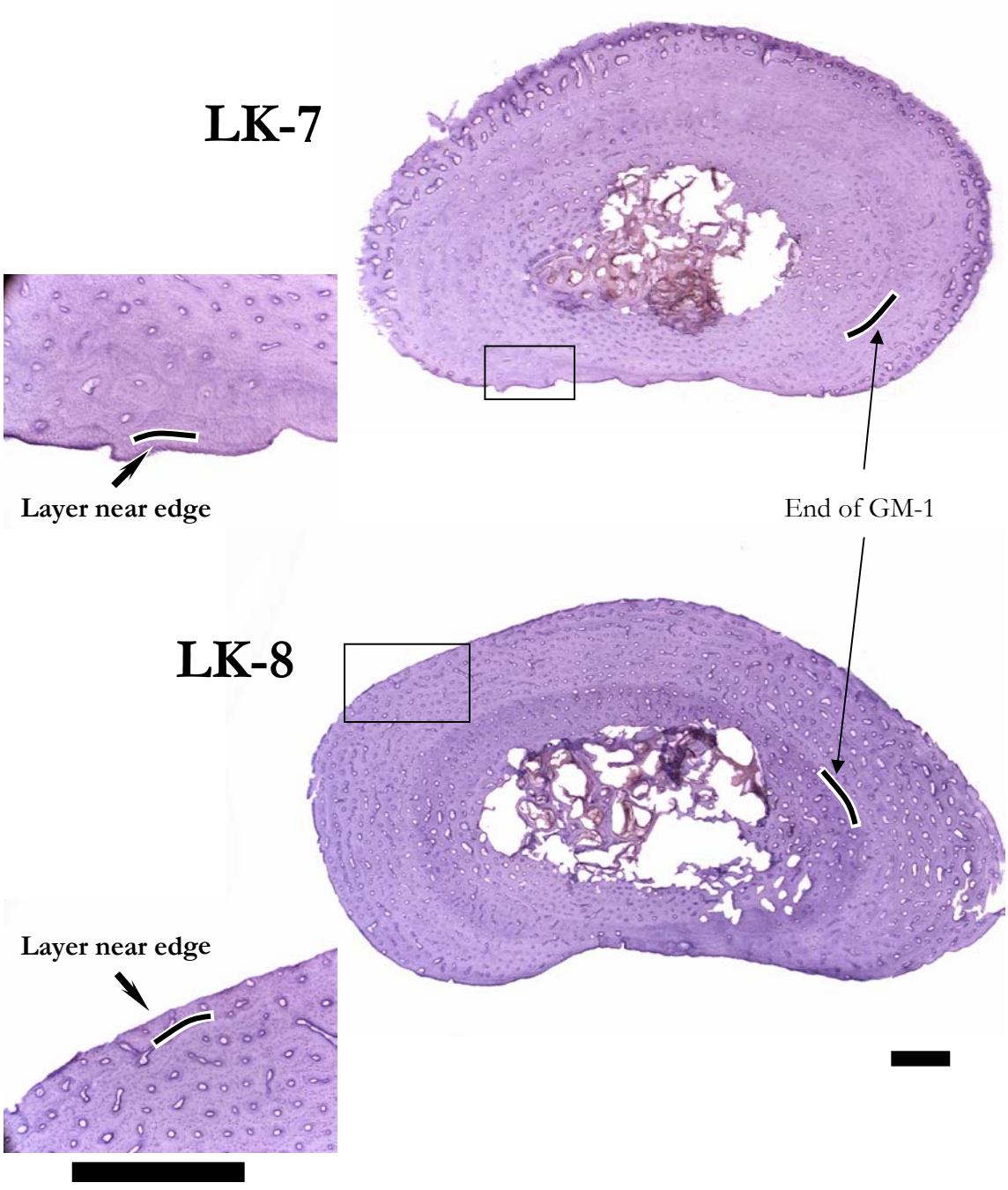


Figure 2.3. Images of humeri cross-sections of coded wire tagged Kemp's ridleys, GM-1 refers to growth mark one. Black bars represent 1mm in length; LK-4, 1.27 years old and LK-5, 2.37 years old.

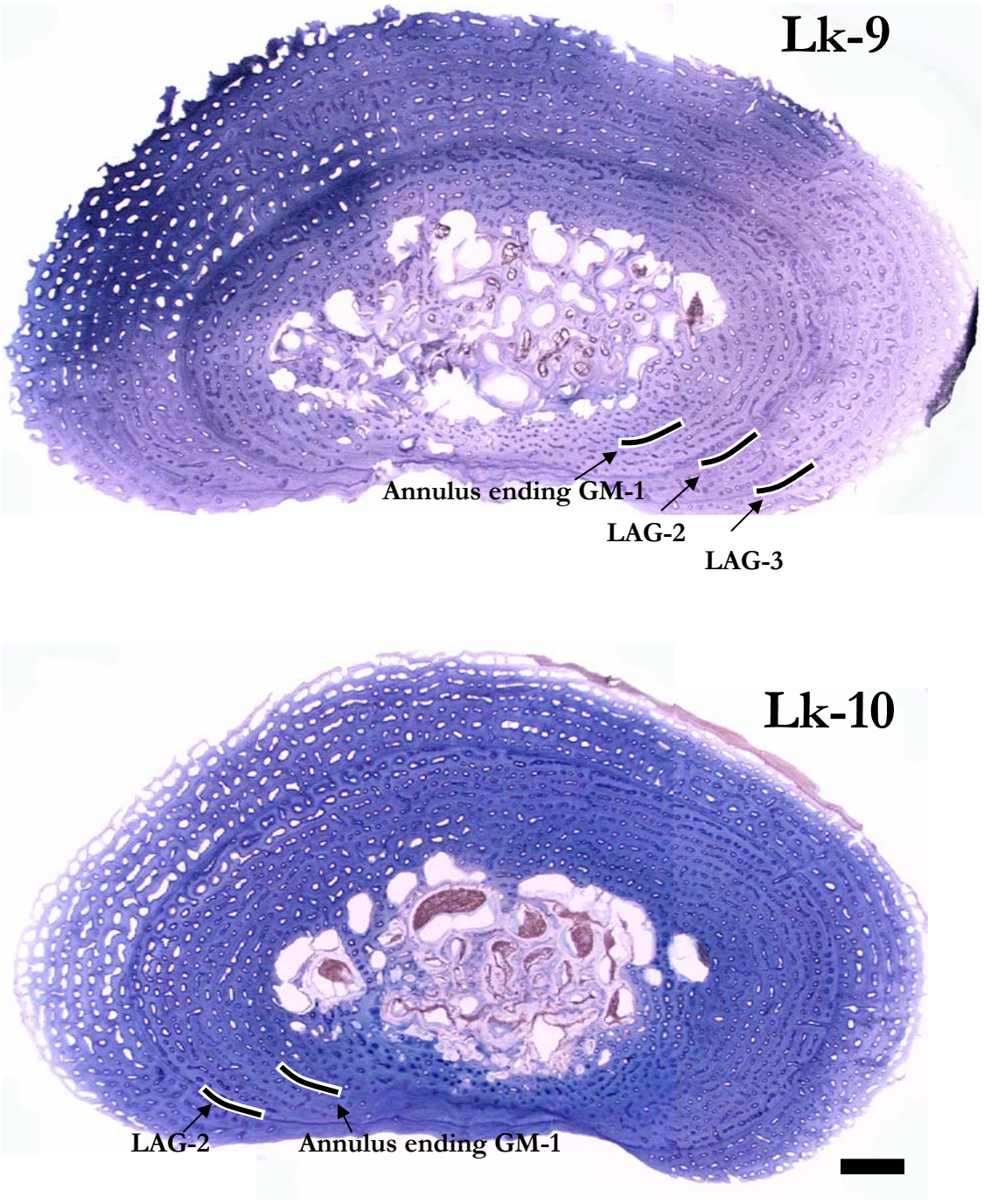


Figure 2.4. Images of humeri cross-sections of coded wire tagged Kemp's ridleys, black bars represent 1mm in length, curved black lines outlined in white highlight LAG's or annuli ending growth. LK-9, 3.25 years old; LK-10, 2.0 years old.

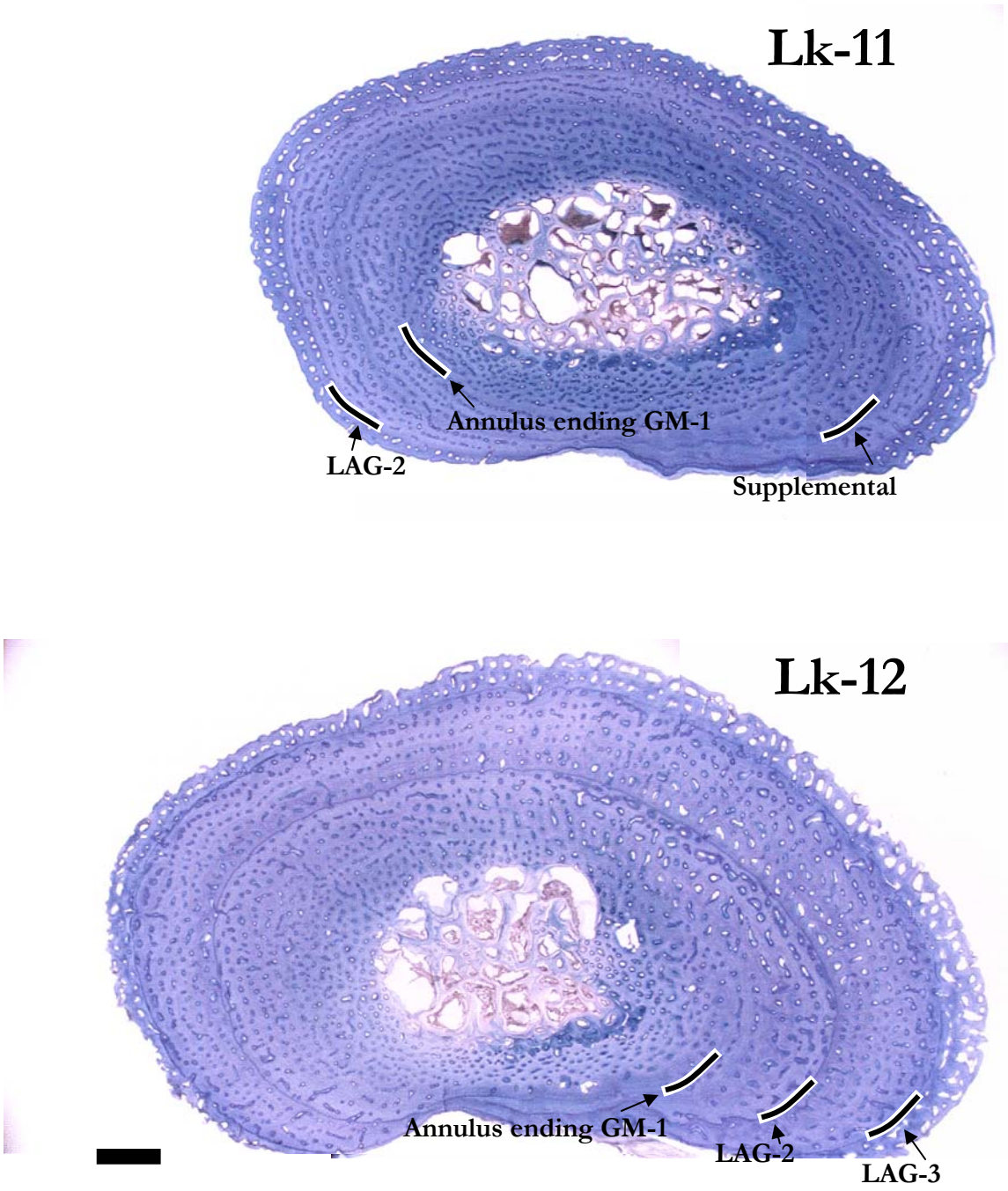


Figure 2.5. Images of humeri cross-sections of coded wire tagged Kemp's ridleys, black bars represent 1mm in length, curved black lines outlined in white highlight LAG's or annuli ending growth. LK-11, 2.75 years old; LK-12, 3.0 years old.

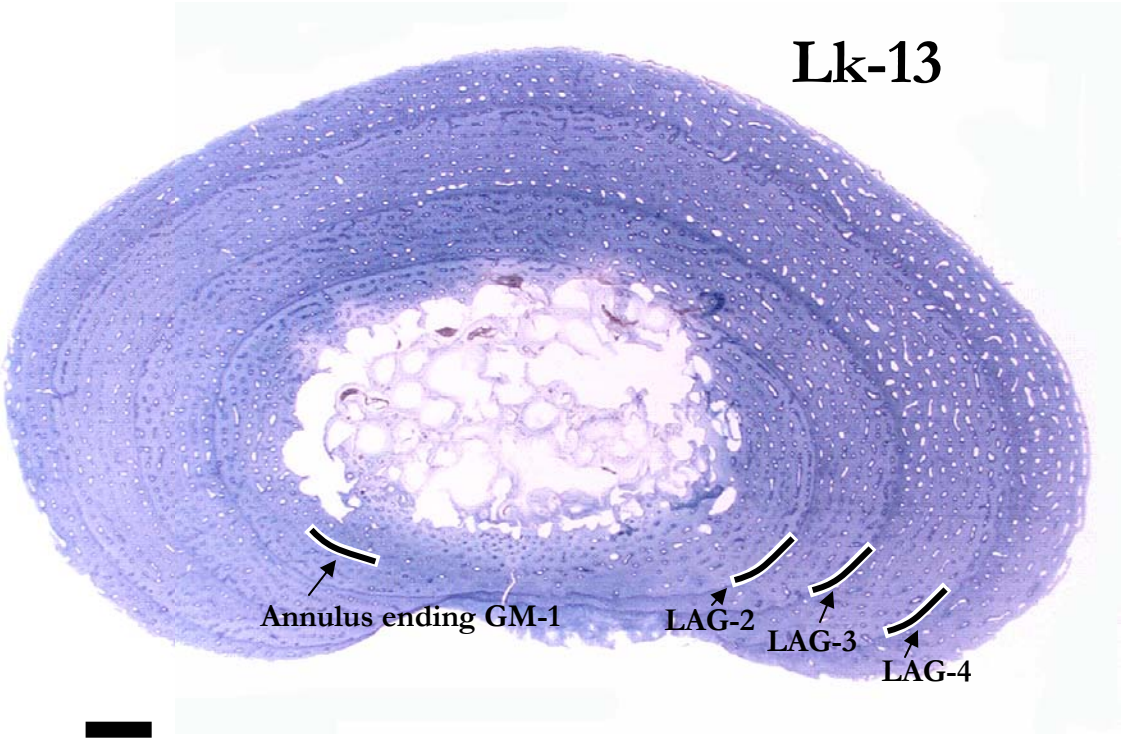


Figure 2.6. Image of humeri cross-sections of coded wire tagged Kemp's ridleys, black bar represent 1mm in length, curved black lines outlined in white highlight LAG's or annuli ending growth. LK-13, 4.75 years old.

marks, which can only be interpreted as a supplemental lines given the age of the animal, appeared between growth mark one and the outer edge of the bone in LK-6 and LK-10 (Fig. 2.2 & 2.5). Specimens LK-7 and LK-8 were difficult to interpret. The LAG representing the end of growth mark 2 appeared to be very close to the outer edge of the bone cross-section in both of these samples, however, there should have been a full growing season between the completion of growth mark 2 and their death in the fall. Both of these animals were recovered as dead strandings in a mass cold stun event that took place in Cape Cod, Massachusetts, USA in the fall of 1999. Humerus cross-sections from LK-9 showed a poorly defined annulus ending growth mark one followed by two well defined LAGs ending the second and third growth marks (Fig. 2.4). Without prior knowledge of this animal's history I accurately aged it at 3.25 years based on growth mark counts and stranding date. Similarly, LK-10 to LK-14 all displayed poorly defined annuli ending the first growth mark but well defined LAGs demarking the end of subsequent growth marks (Fig. 2.4 – 2.6). Each of these animals was aged accurately interpreting the placement of the first annulus and counting the number of LAGs All of these animals, LK-9 through LK-13, demonstrated clearly that well-defined LAGs were deposited at the end of year two, providing evidence that any lines between the year one annulus and the year two LAG were supplemental.

Loggerhead (*Caretta caretta)*

The first known-age loggerhead was 29.4 years old. Eleven LAGs can be followed around the circumference of the bone cross-section and an additional nine LAGs can still be seen within the resorption zone in most areas of the bone (Fig. 2.7). On the dorsal side of the cross-section, at least four less-distinct LAGs or annuli can still be observed; these have

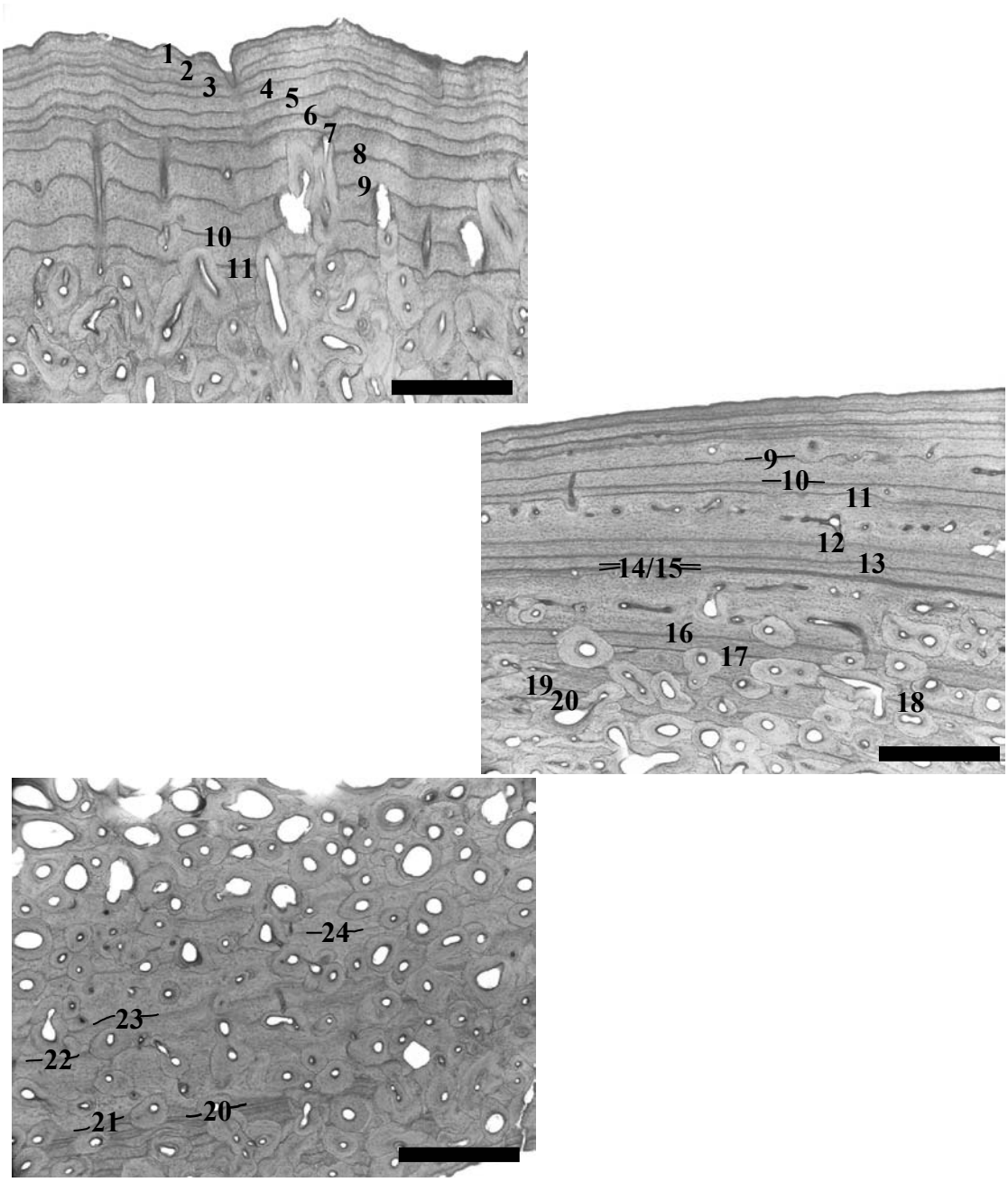


Figure 2.7. Images of different portions of the humerus cross-sections of CC-1. Black bars represent 1 mm in length. Top and middle: outer edge of bone is at the top of the photo; LAGs are labeled with numbers, with low numbers represented the most recently deposited LAGs and higher numbers representing the earlier LAGs. Bottom: outer edge of bone is towards the bottom of the photo.

been resorbed in all other parts of the bone (Fig. 2.7). There has been a great deal of remodeling within the bone and much of the inner portion of the bone has been resorbed. Summing all of these growth marks, I gave a minimum age estimate of 24 years without prior knowledge of the history of the animal. The outermost 20 growth marks contained well defined LAGs that are spaced close together, while the four interior-most visible growth marks contained LAGs or annuli that are spaced further apart (Fig. 2.7). The number of layers completely resorbed was five.

A second known-age loggerhead, CC-2, was eight years old. I gave a minimum estimate of five years and, following Zug et al.'s (1986) linear technique, backcalculated the age to eight years. Just outside of the resorption area was a series of three LAGs very close together (Fig. 2.8). In my initial analysis, I assumed that three GM's so close together could not each be annual and I read them as one year. I re-evaluated this assumption after learning its history. The animal was in captivity for two years then released at 42.7 cm SCL in October 1994. Counting back from the outside of the bone, the outermost of the triplet LAGs would represent spring 1996. I used the Frazer-Lee method (Francis 1990) and the regression equation for bone diameter and SCL (see below, Table 2.5) to estimate size at the diameter of the outermost of the triplet LAGs (I was unable to get an accurate measurement of the innermost LAG). This gave a value of 47.9cm SCL. Given the evidence, our best interpretation of this bone section is that the innermost of the triplet LAG indicates release and therefore is not an annual mark. The next LAG was likely the following spring (1995) and is likely an annual mark. The third of the closely spaced LAGs likely represents spring 1996, indicating that the animal did not grow significantly in its first year in the wild (Fig. 2.8). Following the three closely spaced LAGs, there were 4 additional indistinct LAGs or

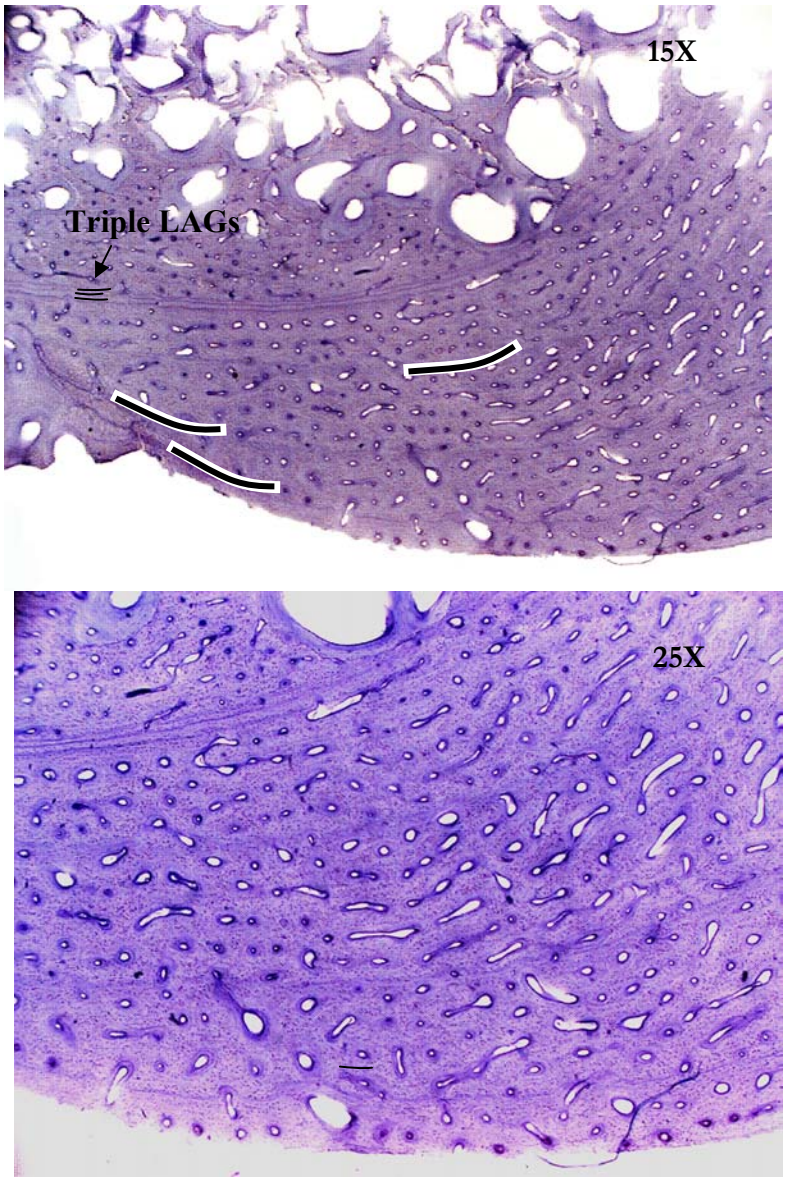


Figure 2.8. Images of the same section of the humeri cross-section of CC-2, taken at different magnifications. Outer edge of bone is towards the bottom of the photos. Top image shows triple LAGs and three diffuse LAGs. Bottom is a higher magnification and shows the LAG at the very edge of the bone (bottom of photo).

annuli that represent the remaining years at large. The outermost of these was very close to the edge of the bone, indicating that the animal did not grow much
if at all during the last summer of its life.

The regression equation for humerus width on carapace length has a slope of 2.38. This relationship approximates carapace growth from bone growth reasonably well (Table 2.4). The greatest deviation was for CC-5 which was overestimated by 3.13 cm, however, as this was over a three year period, this equates to about 1cm/yr overestimation. In all instances, growth was overestimated, however, this is expected as I had to use the LAG most representative of the time of tagging for the bone measurements. In most cases, the assumption of one LAG deposited per year yielded the closest approximation to observed growth as compared to the assumptions of LAG deposition twice per year or every other year (Table 2.4).

Indirect validation of annual growth marks

For Kemp's, there was a significant increase in the amount of bone deposited after the last LAG from June 20 to November 30 (Fig. 2.9; $p<0.005$). The LAGs near the outer edge of the bone are fully visible in strandings that occur after 20 June. This was likely because a certain amount of bone formation must occur following the LAG before it can be discerned from the edge. There was not a significant relationship between bone growth and date from December 1 to June 19 ($p=0.27$). The slope of this regression is very close to zero ($b=-0.003$), indicating no trend, either increasing or decreasing, in the amount of bone deposited during this time (Fig. 2.9).

Validation of the relationship between LAG diameter and body size

The regressions of the eight morphometric measurements of loggerhead and Kemp's

Table 2.4. Summary of the results for the wild tagged and released loggerheads.

ID:	CC-3	CC-4	CC-5	CC-6	CC-7
Initial Tag Date:	11/21/95	11/21/95	6/5/98	6/29/98	7/23/99
Strand Date:	6/7/98	10/18/98	4/2/00	11/29/01	1/15/02
Initial SCL:	62.1	50.6	56.3	61.8	59.0
Final SCL:	72.7	57.6	58.6	70.8	59.6
Growth since initial capture:	10.6	7.0	2.3	9.0	0.60
LAG used to estimate tag date	Spring '96	Spring '96	Spring '98	Spring '98	Spring '99
Estimated growth, assumption of 1 LAG/yr	11.42 (+0.82)	10.13 (+3.13)	2.68 (+0.38)	11.22 (+2.22)	0.61 (+0.01)
Estimated growth, assumption of 2 LAG/yr	25.04 (+14.44)	29.86 (+22.86)	7.40 (+5.1)	23.92 (+14.92)	15.40 (+14.8)
Estimated growth, assumption of 0.5 LAG/yr	4.70 (-5.90)	4.20 (-2.80)	2.68 (+0.38)	2.32 (-6.68)	0.29 (-0.31)

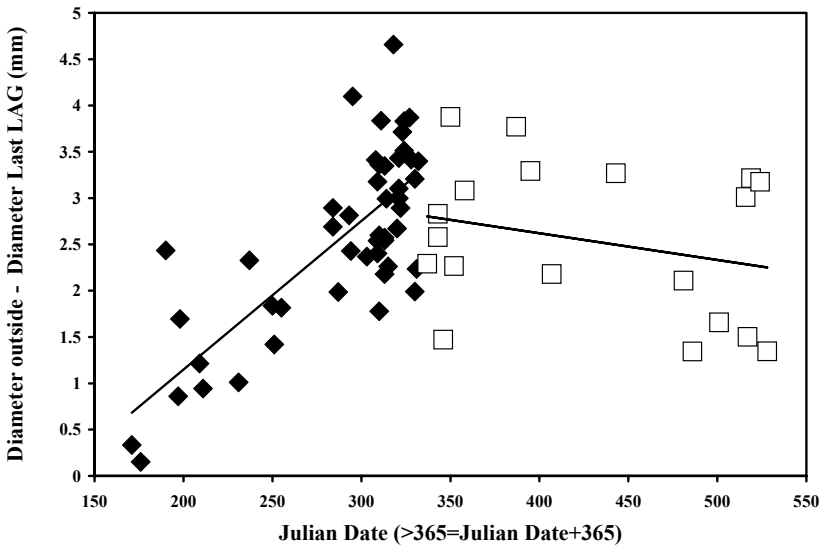


Figure 2.9. Julian date of stranding plotted against the amount of bone deposited peripherally to the last LAG in Kemp's ridleys (N=76). Dates on x-axis equate to 20 June through 19 June. Solid lines represent linear regressions which were run separately for 6 months, 20 June to 31 November and 1 December to 19 June. The regression for the first six months was significant ($P<0.006$) while the regression for the second six months was not sigificant ($P=0.27$).

humeri against carapace length (SCL) revealed high correlations between bone dimension and body size (Table 2.5). The diameter at the distal end of the insertion scar of the deltopectoral muscle on the ventral side (the site where I removed cross-sections for skeletochronology) and the body length of the animal were highly correlated. Applying this regression equation to the diameters of 25 additional loggerhead humeri I found significant relationship ($P<0.005$) between carapace length estimated from the width of the bone at the cross-sectioning site and the observed carapace length. The slope of the regression, 0.82, was significantly different from a slope of one ($p=0.006$; Fig. 2.10). For Kemp's, the relationship between estimated and observed carapace length was also significant ($P<0.005$) and the slope, 1.06, was not significantly different from one ($p=0.38$; Fig. 2.10).

DISCUSSION

Annual periodicity in growth marks

The results supported annual deposition of growth marks in loggerhead and Kemp's ridley sea turtles. The headstarted Kemp's ridleys in particular highlighted the likelihood of annual marks. These animals were in the wild for at least three years and displayed sharp and regularly spaced LAGs that were consistent with the known-age of the animals. The indirect validation results for this species highlighted the cyclic nature of bone growth with increasing bone deposition occurring from late spring/early summer to fall and no bone deposition occurring from fall to spring. From this I inferred that LAGs form annually in the spring for Kemp's that strand along the mid- to southeast U.S. Atlantic coast and are visible at the edges of the bones by late spring to early summer. The results from the CWT Kemp's ridleys emphasized the difficulties in interpreting early growth marks (Fig. 2.6).

Table 2.5. Regressions equations and fit statistics from correlations between dimensions of the humerus and notch-to-tip straight carapace length (cm) in loggerhead and Kemp's ridley sea turtles. All F statistics are significant at P<0.005.

Humeral Measurement	Model Equation	SE Slope	F	r^2
Loggerheads N=243				
Maximal Length (mm)	SCL=0.44*ML + 5.97	0.0064	**4814**	0.95
Longitudinal Length (mm)	SCL=0.47*LL + 4.85	0.0064	**5381**	0.96
Proximal Width (mm)	SCL=1.06*PW + 7.31	0.015	**4857**	0.95
Deltopectoral Crest Width (mm)	SCL=1.69*DPPW + 6.04	0.026	**4069**	0.94
Site of Sectioning Width (mm)	SCL=2.38*SW + 5.48	0.037	**4110**	0.94
Site of Sectioning Thickness (mm)	SCL=4.13*ST + 11.62	0.080	**2682**	0.92
Distal Width (mm)	SCL=1.28*DW + 5.43	0.021	**3684**	0.94
Mass (g)	LN(SCL)=0.30*LN(M) + 2.94	0.0022	**18905**	0.99
Kemp's ridleys N=262				
Maximal Length (mm)	SCL=0.43*ML + 4.69	0.0040	**10970**	0.98
Longitudinal Length (mm)	SCL=0.47*LL + 3.11	0.0039	**14772**	0.98
Proximal Width (mm)	SCL=1.12*PW + 4.39	0.010	**12390**	0.98
Deltopectoral Crest Width (mm)	SCL=1.69*DPPW + 3.35	0.017	**10200**	0.98
Site of Sectioning Width (mm)	SCL=2.48*SW + 2.74	0.033	**5715**	0.96
Site of Sectioning Thickness (mm)	SCL=4.16*ST + 4.79	0.072	**3306**	0.93
Distal Width (mm)	SCL=1.36*DW + 0.227	0.013	**11435**	0.98
Mass (g)	LN(SCL)=0.30*LN(M) + 2.89	0.0023	**16305**	0.98

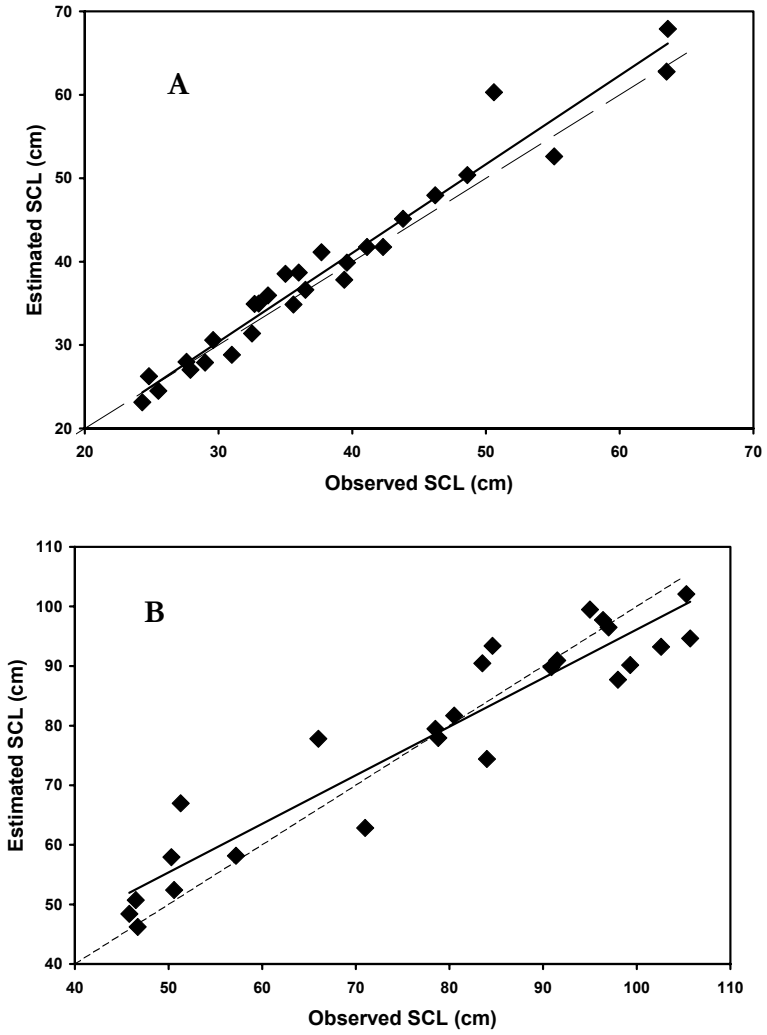


Figure 2.10. Carapace length of (a) Kemp's ridley (N=28) and (b) loggerhead (N=25) sea turtles were estimated from humeri diameters. Solid line indicates the least-squares linear regression on the data, dotted line represents the hypothesized one-to-one relationship between estimated and observed carapace length. The slope of the regression for Kemp's ridleys (a) is not significantly different from one (P=0.38) while the slope of the regression for loggerheads (b) is significantly different from one (P=0.006).

From these animals I concluded that in general Kemp's deposit a poorly defined annulus in their first year, and well-defined LAGs starting with the end of the second year and in subsequent years. For loggerheads, only CC-2 spent any time in the wild. The number of growth marks deposited after the animal was released (based on backcalculated size) was consistent with the number of years for which the animal was at large, considering that the first mark was deposited at release (Fig. 2.8). This indicated that not less than one growth mark was deposited per year, but that additional or supplemental LAGs or annuli indistinguishable from annual lines may be deposited under extreme conditions, such as release into the wild, but in this case it was not frequent enough to have a serious impact on age estimates.

For the life-time captive animal, CC-1, our estimated minimum age was five years shorter than the actual age of 29.4, which clearly demonstrated that not more than one growth mark was deposited per year. With the relatively large size of the sea turtle humerus, in comparison to phalanges of amphibians, rapprochement did not appear to be a problem in discerning the LAGs. This bone was similar in appearance to adult wild loggerheads and Kemp's with rapprochement of the peripheral LAGs and resorption of most of the interior growth marks. Although accurate age estimates cannot be made of these bones through skeletochronology, if rapprochement correlates to the timing of sexual maturity, counts of the compressed growth marks can provide valuable information on post-reproductive longevity. This information can be combined with average age at reproductive maturity in piecing together the life history of sea turtles.

The results of the five loggerheads from the mark-recapture study also supported the deposition of one growth mark per year over assumptions of two per year or one every

other year. In addition, these results verified that measurements of growth mark diameters can be used to estimate somatic growth rates in terms of carapace length.

As evidenced in the literature survey (Table 2.2), most studied species of reptiles and amphibians deposit growth marks within their bones. For some of these species, the annual nature of the growth marks has been validated. For others, it is in keeping with their ecology that the marks must represent annual events (Castanet et al. 1993). Growth marks observed in loggerhead, Kemp's ridley and green sea turtles are similar in structure to those observed in other species of reptiles and amphibians (Zug et al. 1986, Klinger and Musick 1992, Klinger and Musick 1995, Zug et al. 1995, Zug and Parham 1996, Parham and Zug 1997, Zug et al. 1997, Zug and Glor 1998, Coles et al. 2001, Zug et al. 2002). Based on previous studies of reptiles and amphibians, validation studies on sea turtles, and the evidence presented in this paper, I assert that growth marks in bones of sea turtles are likely deposited with a primarily annual periodicity.

Given these results, on the surface it seems contradictory that in two validation studies, annual growth marks could not be confirmed. For serpentine species, Collins and Rodda (1994) inject brown snakes with a fluorescent marker and keep them in captivity for one year under two different feeding regimes. Five or six growth marks varying in distinctness are identified beyond the fluorescent marks in bone cross-sections. Statistical analyses suggest these marks relate to shedding events. It is unclear if the growth-mark pattern prior to captivity is similar to what is seen after the fluorescent mark. The forced feeding component of this study may induce higher growth rates than would be found in nature, causing the shedding events to appear as histological marks in the bone.

In a sea turtle study, Bjorndal et al. (1998) did not find growth marks in the humeri

of green sea turtle bones. They suggest that the tropical marine habitat of the study population (approximately 21°07'N) allows for continual activity and growth, inhibiting growth mark formation. However, growth marks have been clearly demonstrated in green sea turtles from the coastal waters of Florida, USA (approximately 29°N) (Zug and Glor 1998) and Hawaii, USA (approximately 22°N) (Zug et al. 2002). Other studies of reptiles and amphibians report distinct growth marks in species with year-round activity (Patniak and Behera 1981, Estaban et al. 1996, Guarino et al. 1998).

Growth mark interpretation

Three interpretations of double and bifurcating LAGs have been suggested. First, if double LAGs appear frequently in individual bones and throughout the sample, they likely indicate an ecology that has two growth cycles per year (Castanet et al. 1993). In this case the two LAGs are distinct from each other over the entire bone cross-section. This pattern was observed in the newt, *Triturus marmoratus*, living at a high altitude where the animals had both winter and summer dormancy periods (Castanet and Smirina 1990, Caetano et al. 1985, Caetano and Castanet 1993). A second interpretation of double LAGs is that they result from a brief interruption of hibernation (Hemelaar and van Gelder 1980). Hemelaar and van Gelder (1980) suggest that little bone deposition would occur in this instance and the layers would not be distinct from each other over the entire bone, thus giving the appearance of a bifurcating LAG. The third interpretation of the double LAG is that they result from extreme decreased growth over the active period, which places annual LAGs very close to each other and in some cases appear to merge (De Buffrenil and Castanet 2000).

With the first two interpretations, a double or bifurcating LAG would be counted as

one for the purposes of age estimation, while the third interpretation would necessitate counting each LAG or bifurcating branch separately. Coles et al. (2001) recovered a dead stranded adult loggerhead 8.25 years after it had been injected with oxytetracycline. In cross-sections of the humerus, they found seven LAGs following the tetracycline mark, counting a bifurcating LAG as one, and consider this the appropriate number of LAGs if the LAGs are annual. The animal was marked on 20 June 1989 and recovered dead on 22 September 1997. Making the reasonable assumption that, as with Kemp's ridleys from the same region, the LAGs form in the spring, there should have been eight LAGs deposited after the tetracycline mark, not seven (each representing spring of '90, '91, '92, '93, '94, '95, '96 and '97). If the LAGs mark annual events, the bifurcating mark in this bone should be counted as two LAGs.

Our observations of known-age material also supported the interpretation of double and bifurcating LAGs being counted individually. The CWT Kemp's ridleys, samples LK-7 and LK-8, displayed LAGs near the outer edge of the bone but with a small amount of bone deposited afterwards. These animals were each 2.25 years old with one- year marks but no LAG or annuli other than the peripheral LAGs that would represent year two. Sample LK-9 clearly indicated that a LAG is deposited at the end of the second GM. The indirect validation results demonstrated that LAGs were visible in bone tissue by late spring or early summer. It seemed that the LAGs at the outer edge of the CWT bones were the year 2 LAGs and that very little growth occurred over the subsequent growing season. Both of these animals were recovered as dead strandings resulting from a major cold stun event in Cape Cod, Massachusetts, USA in 1999, hence their growth rates may have been anomalous in their last year of life. Had these animals survived the cold stun event, they

would have deposited year three LAGs very close to year two, giving the appearance of a double or bifurcating LAG. Results from CC-2 also supported the interpretation of counting double or bifurcating LAGs individually. I initially considered the triple LAGs just as a single mark, however, after learning the animal's history, it became clear that two of the LAGs were annual. In addition, the last LAG in this animal was very near the outer edge of the bone. Again, as with the CWT Kemp's, had this animal survived another year it would have had two annual LAGs very close to each other.

Wild loggerhead growth rates have been monitored in an ongoing mark-recapture study in Pamlico and Core Sounds in North Carolina, USA (Braun-McNeill et al. in prep). They obtained growth rates for 35 juvenile loggerheads between 50 and 75.8 cm SCL at initial capture. Each of these animals had been at-large for multiples of one year intervals, plus or minus one month. Of the 35 growth rates, five of them show an annual increase of 0.3 cm or less in straight carapace length (J. Braun-McNeill – personal communication). Using the equation for width at sectioning site from Table 3, the increase in bone diameter for these five animals would be 0.13mm or less, which places the LAGs very close together.

I observed multiple splitting LAGs in CC-1. Francillon-Viellot et al. (1990) examined different bones from the same animal to determine that each thin LAG comprising a split LAG should be counted individually. In accordance with this observation and our previously described observations on bifurcating LAGs, I counted each individual LAG emerging from a splitter as one. As the LAG count was close to the actual age of the animal, this appears to have been the appropriate interpretation.

Supplemental lines may form as a result of temporary environmental stressors such as droughts. In support of this, Rogers and Harvey (1994) note a supplemental line in 11 of

43 specimens of the toad, *Bufo cognatus*, and in 10 of those animals, the supplemental line is within a growth zone that corresponds to a drought year. Most skeletochronology studies that note the presence of supplemental lines indicate that they are easily identified as such because they are less distinct and do not appear around the entire circumference of the bone. In general, the same has been observed in sea turtles. Supplemental lines do appear but are easily differentiated from LAGs. An exception to this is the presence of supplemental marks in one-to-two-year-old Kemp's ridleys. These marks were only able to be identified as such from the observation of known-age animals. In addition, there appears to be a supplemental line in CC-2 that represents when the animal was released.

Factors Influencing LAG Morphology

Much of the herpetological literature attributes the formation of LAGs to low metabolism and no growth associated with seasonal climate changes. However, because LAGs, or 'rest lines' are found almost universally in bone and teeth of vertebrate species (Klevezal 1996), Castanet et al. (1993) propose that the formation of LAGs is likely to be endogenous while still potentially synchronized to environmental conditions.

Our findings supported that the deposition of annual resting marks appeared to be under endogenous control while the morphology of the resting mark may be influenced by growth rates or the local conditions to which the animal is exposed. In Kemp's ridleys, the layer completing the first growth mark is an indistinct annulus. Very young animals are likely experiencing high growth rates and there may not be a complete cessation of growth in the first year, resulting in an indistinct annuli. By year two, Kemp's appeared to deposit well-defined LAGs at the end of growth marks. The known-age loggerhead, CC-2, was released, recaptured, and recovered dead in the Gulf of Mexico, indicating that it may have

been resident in these waters. The growth marks deposited after this animal was released were much more poorly defined than growth marks observed in loggerheads that strand dead along the mid-Atlantic coast, indicating that latitude and water temperatures may influence the morphology of the growth marks.

In other species of reptiles, the distinctness of the LAG is related to the amount of activity of the animal during periods of hibernation (Waye and Gregory 1998, De Buffrenil and Castanet 2000). Waye and Gregory (1998) raised hatchling garter snakes in captivity, manipulating the hibernation periods. Snakes that undergo normal hibernation periods have one distinct LAG the following spring. Snakes that are kept out of hibernation have several indistinct 'annuli' type structures by the following spring, while snakes that are aroused from hibernation, fed ad libitum, then returned to hibernation display 2 indistinct 'annuli' the following spring. Thus, even under severely abnormal conditions of captivity without hibernation or interrupted hibernation, a faint indication of a resting line in the form of several indistinct annuli is still deposited at the time when a LAG would normally have been deposited, giving justification for endogenous control. Schauble (1972) did an experiment in which she amputated limbs from the newt, *Notophthalmus viridescens*, at different times of the year and observed the regeneration rates. She found that regeneration rates were significantly higher in the spring/early summer months, followed by summer, late summer, early fall and winter, respectively. As temperature, light levels and food availability were controlled, these factors could not have played a role in the regeneration rates. Schauble (1972) suggests that the results imply the existence of an internal biological rhythm, either endocrine or nonendocrine in nature, controlling a spring surge in bone growth.

Esteban et al. (1996) did a skeletochronological study of the Iberian water frog (*Rana perezi*). These frogs are from a subtropical region where they continue to be active throughout the year with no distinct periods of aestivation, although their activity patterns switch from diurnal during the winter months to nocturnal in the warmer months. They find distinct growth marks composed of a zone and either a LAG or annuli in 93% of their specimens. In the other 7% (N=7) they find either extremely diffuse marks or no marks at all. Based on these 7 animals and the individual variation observed in the rest of the sample, Esteban *et al.* (1996) state that the structure and spatial pattern of growth marks is dictated mainly by external factors rather than an endogenous, genetic control. The structure of the growth marks, particularly the resting mark, appears to be influenced by external factors. However, the fact that these animals remain active throughout the year and 93% of the sample deposite readily identifiable growth marks provides a strong indication of endogenous control over the spatial pattern created by the formation of one growth mark per year.

Resorption

The loss of the early growth marks due to endosteal resorption and remodeling of the interior region of the bone is a limiting factor in the application of skeletochronology to sea turtles. Based on our findings, it was possible to accurately age juvenile Kemp's ridleys up to at least 5 years based on GM counts and this may be true for other sea turtle species with the possible exception of the leatherback (Zug and Parham 1996). As sea turtles have distinct life history stages, I suggest that to age a population of sea turtles, one must acquire an ontogenetic series of samples spanning all sizes and stages. Average duration can be determined for each ontogenetic stage and the approximate age of older animals with

extreme resorption can be estimated. As growth mark patterns appear to mimic somatic growth rates, once growth through each life history stage is understood, backcalculation techniques to estimate the number of layers lost can be used.

For many species, skeletochronology is not a perfect method of age estimation. As growth marks are histological expressions of variations in rates of osteogenesis (Castanet et al. 1993), external factors and individual variation influence the appearance of the marks (Castanet et al. 1993, Esteban et al. 1996, Waye and Gregory 1998, Esteban et al. 1999). Endosteal resorption also serves to confound this technique and is the primary difficulty in the application of the technique to sea turtles. However, the evidence presented here gave strong support to the concept that growth marks were deposited on an annual basis in sea turtles and that the spatial pattern of the growth marks corresponded to the growth rates of the animal. The growth marks therefore provide invaluable information on age and growth that cannot otherwise be easily obtained and the technique is valid and appropriate to the study of sea turtles.

CHAPTER 3

SUGGESTION OF AN ONTOGENETIC HABITAT SHIFT IN THE GROWTH OF JUVENILE LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*)

INTRODUCTION

There are limits to the body size of an individual that can be supported by a particular habitat. Organisms that span a large range of body sizes during their ontogeny often alter their habitat and diet as they grow in order to obtain optimal growth rates while minimizing predation mortality (Werner and Gilliam 1984, Werner 1988, Dahlgren and Eggleston 2000). Animals may use nutritionally sub-optimal habitats to minimize the risk of predation until a size refuge is reached, at which point an ontogenetic habitat shift occurs.

These ontogenetic habitat shifts often reflect a growth advantage in the new habitat as demonstrated for bluegill sunfish (*Lepomis macrochirus*) and Nassau grouper (*Epinephelus striatus*) (Werner et al. 1983, Werner and Hall 1988, Dahlgren and Eggleston 2000). The pelagic zone of lakes is a much more profitable habitat for small bluegills as opposed to vegetated littoral zones, but small bluegills are confined to feeding in littoral zones when major predators, largemouth bass (*Micropterus salmoides*), are present, trading off higher growth rates for decreased mortality until a size refuge is reached (Werner et al. 1983, Werner and Hall 1988). Results from caging experiments demonstrate that small Nassau grouper which typically use macroalgae habitats have significantly higher growth rates but also significantly higher predation rates in the postalgal habitats used by larger grouper (Dahlgren and Eggleston 2000).

Juvenile loggerhead sea turtles undergo at least one major ontogenetic habitat shift, the transition from a pelagic to a benthic habitat. For loggerheads nesting in the southeast United States, hatchlings and post-hatchlings are caught up in the Gulf Stream and eventually become entrained in the North Atlantic gyre (Carr 1987, Hays and Marsh 1997, Lohmann et al. 2001). Now juveniles, they remain pelagic, completing a full transatlantic migration before returning to the western North Atlantic (Carr 1987, Bolten et al. 1998). After juvenile loggerheads return to the western North Atlantic, they enter a benthic life stage, inhabiting coastal waters of the mid- and southeast United States for the duration of their life (Carr 1987). Based on Werner and Gilliam's (1984) theory of ontogenetic habitat shifts, I would expect an increase in growth rates in loggerhead sea turtles following the shift from pelagic to benthic habitats.

I have been collecting humeri from dead, stranded carcasses and analyzing growth marks in cross-sections of the bones (Zug et al. 1986, Klinger and Musick 1992, Klinger and Musick 1995, Zug et al. 1995, Parham and Zug 1997). Distinct growth marks are annual and so can be used to age turtles (Klinger and Musick 1992, Coles et al. 2001, Chapter 2). The spatial pattern of the growth marks directly correlates with somatic growth rates, that is, close spacing of marks indicates slow growth while wide spacing of marks indicates fast growth (Chapter 2).

I hypothesized that loggerheads would experience a growth benefit associated with the pelagic-to-benthic shift and that this shift can be detected from the growth mark spacing in cross-sections of bone tissue. Similar settlement marks have been observed in the daily growth increments of the tropical marine goby (*Bathygobius coalitus*, Shafer 2000). If I observe wider spacing of the post-settlement growth marks, I also hypothesized that the

shift in spacing of the growth marks would correlate to a diet shift from pelagic to benthic foods. As the stable isotope ratios found in bone and teeth are representative of an animal's diet at the time bone or dentin tissue is deposited, stable isotopes from bone and tooth collagen can provide information on migration patterns and trophic relationships in marine mammals, turtles, and sea birds (Hobson 1993, Godley et al. 1998, Hobson and Sease 1998, Burton and Koch 1999, Walker and Macko 1999, Walker et al. 1999). Hence, I used stable isotope ratios of carbon and nitrogen within the bone cross-sections to establish if a shift in diet occurred simultaneously with a growth rate shift, indicating that the growth rate shift occurred at the time of settlement. Diameters of growth marks were used as proxies for somatic growth to observe growth dynamics before and after the growth rate shift.

MATERIALS AND METHODS

Humeri Preparation

I received front flippers from dead loggerheads stranded along the coasts of North Carolina, Virginia and Maryland. For most of the turtles, straight carapace length (SCL) was recorded, measured as standard straight-line length from the nuchal notch to the posterior end of the posterior marginal. If only curved carapace length (CCL) was recorded, it was converted to SCL using the following regression equation generated from 203 paired measurements from strandings records for loggerheads from 3.8 to 114.7cm SCL (r^2 = 0.966; $P<0.001$):

$$SCL = 0.923*CCL + 0.189.$$

A random subsample of 84 specimens between 43-81 cm SCL was selected for this study. For each, I dissected out the humerus then cleaned off the soft tissue by flensing and boiling. A whole 2-3mm thick cross-section was taken just distal to the deltopectoral ridge

from each of the dried bones (Chapter 2). These were preserved in formalin for analyses of growth marks. For 23 of the specimens, an additional 1mm thick cross-section was taken and saved for stable isotope ratio analysis.

Growth Mark Analysis

The 2-3mm thick sections of bone were decalcified in acid using a commercial preparation, RDO (Apex Engineering Products Corporation, Plainfield, IL, USA). Once decalcified, they were thin-sectioned to 25 μm using a freezing-stage microtome. Thin-sections were stained using Erlich's Hematoxylin diluted 1:1 with distilled water (Klevezal 1996). Digital images of the stained sections were taken at 10x magnification. Visible growth marks were counted and measured from these images. In a thin-section of bone there are thin lines that stain dark, called lines of arrested growth (LAGs), alternating with broad lighter stained zones, generally considered to represent the region of active growth (Castanet et al. 1993). Together, one LAG and one broad zone make up a skeletal growth mark (GM) and represent one year of growth (Chapter 2). In a cross-section, the interior LAGs represent growth earlier in life while the most external LAG was the most recently deposited.

Measurements were made of LAG diameters on the lateral axis of the bone, parallel to the dorsal edge. The growth increment associated with a LAG is representative of the amount of growth that took place the year preceding when the LAG was deposited (Fig. 3.1). When an outer LAG diameter was notably larger than the preceding LAG, the preceding LAG was identified as LAG(0).

The SCL measurements from the 84 turtles were plotted against the number of LAGs after LAG(0), which estimated the time, in years, that the animal spent in the

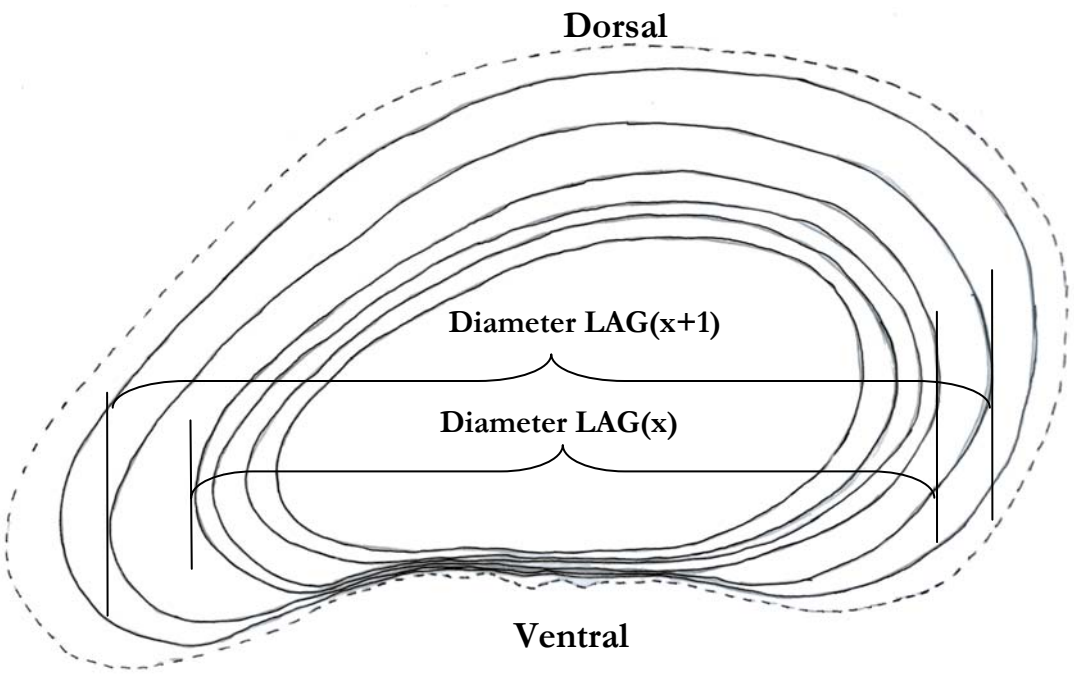


Figure 3.1. Schematic drawing of a humerus bone cross-section. Outer dashed line represents the outside of the bone, solid lines represent LAGs. Growth increment for LAG (x+1) = Diameter LAG(x+1) – Diameter LAG (x).

benthos. This relationship was fit with a least-squares linear regression to estimate the average size at settlement from the y-intercept value.

Stable Isotope Ratio Analysis

In addition to the 23 loggerhead humeri, I collected samples of *Sargassum* and associated fauna from the Gulf Stream off the coast of North Carolina. I also collected several species of nearshore benthic crabs and vegetation plus fresh stomach contents of dead loggerheads. Muscle tissue was isolated from the prey animals and this tissue plus the plant materials were lyophilized then dried at 40° C for stable isotope ratio analysis.

Bone sections were refluxed in distilled dichloromethane prior to isotope analysis to remove lipids. Using the stained thin-sections as a guide, I identified the change in growth mark spacing on the cross-sections and sampled the cross-sections in two locations: internal and external to the change in spacing. The lipid-extracted bone samples and the lyophilized prey and plant specimens were ground to a fine powder and weighed.

Samples were converted to CO_2 and N_2 for isotope analysis using a Carlo Erba elemental analyzer which was coupled to an OPTIMA stable isotope ratio mass spectrometer (Micromass, Manchester, UK). Generally, this conversion is a high temperature combustion involving strong oxidation at high temperature (1020°C), followed by reduction at lower temperature (650°C). The effluent gases are introduced into the mass spectrometer using a continuous flow interface. The stable isotopic ratios are reported as follows:

$$\delta^{N}E = [R_{sample}/R_{standard} - 1]\ 10^{3}\ (‰)$$

where N is the heavy isotope of the element E and R is the abundance ratio of the heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of that element. The standard for carbon is the Peedee Belemnite limestone (PDB) and for nitrogen the standard is atmospheric N_2 (Air) which are assigned $\delta^{N}E$ values of 0.0‰. The reproducibility of the measurement is typically better than ±0.2‰ for the elements using the continuous flow interface on the OPTIMA. The samples were measured against tanks of carbon dioxide and nitrogen gases, which had been calibrated against NBS 22 and atmospheric N_2, respectively. Two-sample t-tests with unequal variances were performed on the stable isotope data from the bone sections to detect significant differences in $\delta^{13}C$ values and $\delta^{15}N$ values before and after LAG(0).

Pelagic Stage Duration in Loggerheads

Due to endosteal bone resorption, it is not possible to directly assess the duration of the pelagic stage in loggerheads from LAG counts. I backcalculated the number of layers lost by applying least-squares linear regression to individual growth trajectories for turtles that had 6 or greater measurable LAGs, including LAG(0) (N=31). I made the assumption that at age 1, loggerheads are 15 cm SCL (Bjorndal et al. 2000b, Bjorndal et al. in review). I converted LAG diameters to estimates of SCL using the regression equation for carapace length on bone section width (Table 2.5 in Chapter 2) to compare pelagic stage growth rates and size-at-settlement.

RESULTS

Growth Mark Analysis

In all 84 bones, I noted a consistent change in the layering pattern of the growth marks. The LAGs were narrowly spaced in the interior of the bone and more widely spaced

on the exterior, with the transition occurring as a sharp change in spacing of the growth marks. For each bone I numbered the LAGs by designating the one just previous to the increase in the layer thickness as LAG(0). LAGs preceding LAG(0) were labeled starting with negative one and counting in toward the center of the bone, and LAGs after it were numbered starting with one and counting out toward the periphery of the bone (Fig. 3.2).

The average growth increments prior to LAG(0) were significantly lower than the average growth increments after LAG(0) (Student's T-test; $P<0.005$; Fig. 3.3). Growth increments at LAG(0) were also significantly lower than the average growth increment at LAG(1) (Student's T-test; $P<0.005$; Fig. 3.3). Regressing the number of LAGs after LAG(0) with reported carapace length at death gave an estimate of 48.5 to 51.1 cm SCL at LAG(0) (mean ± 1 SE; $P<0.05$; Fig. 3.4).

Stable Isotope Ratio Analyses

Bone was sampled for stable isotope analysis just prior to and after LAG(0). Based on two-sample T-tests with unequal variances, the average stable isotope ratios for both carbon and nitrogen were significantly different between the bone sampled internal to LAG(0) and external to it ($P<0.005$, Fig. 3.5). For each bone, the $\delta^{13}C$ values were more negative interior to LAG(0) than exterior to it, with a mean difference of 1.4‰. Also, the $\delta^{15}N$ values of each bone were lower interior to LAG(0) as compared to exterior to LAG(0), with a mean difference of 3.0‰.

The *Sargassum* and *Spartina* vegetation had very similar $\delta^{15}N$ values but *Spartina* had a less negative $\delta^{13}C$ value (Table 3.1). There was no overlap in $\delta^{15}N$ values between the

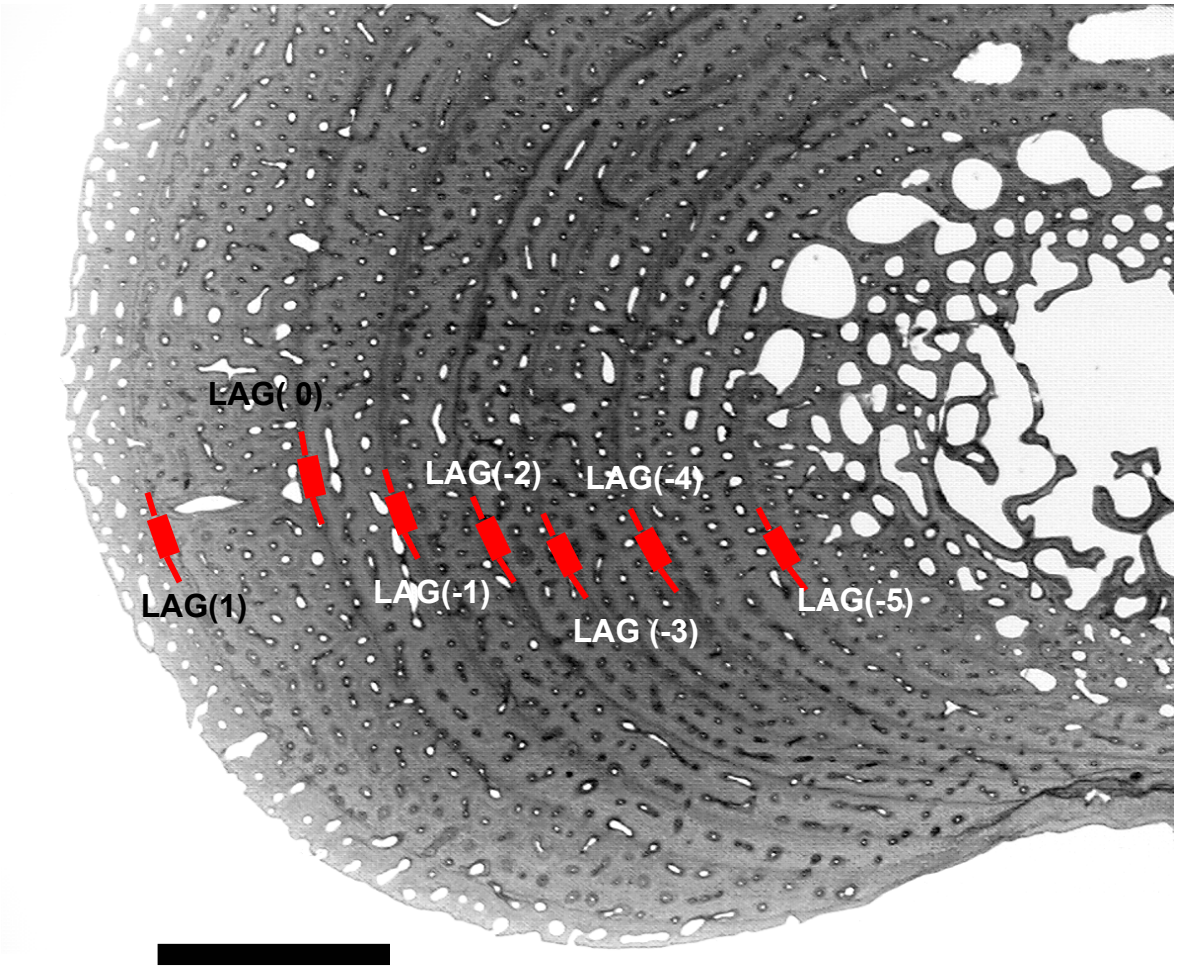


Figure 3.2. Digital image of a partial cross-section of a loggerhead humerus at 10X magnification. Outer edge of bone is to the left of the image and the interior area of resorption is to the right. Black bar represents 1mm. LAG(0) identifies the LAG prior to the shift in growth mark spacing. LAGs interior to LAG(0) were numbered starting with (–1) and counting in while exterior LAGs were numbered with (+1) and counting out

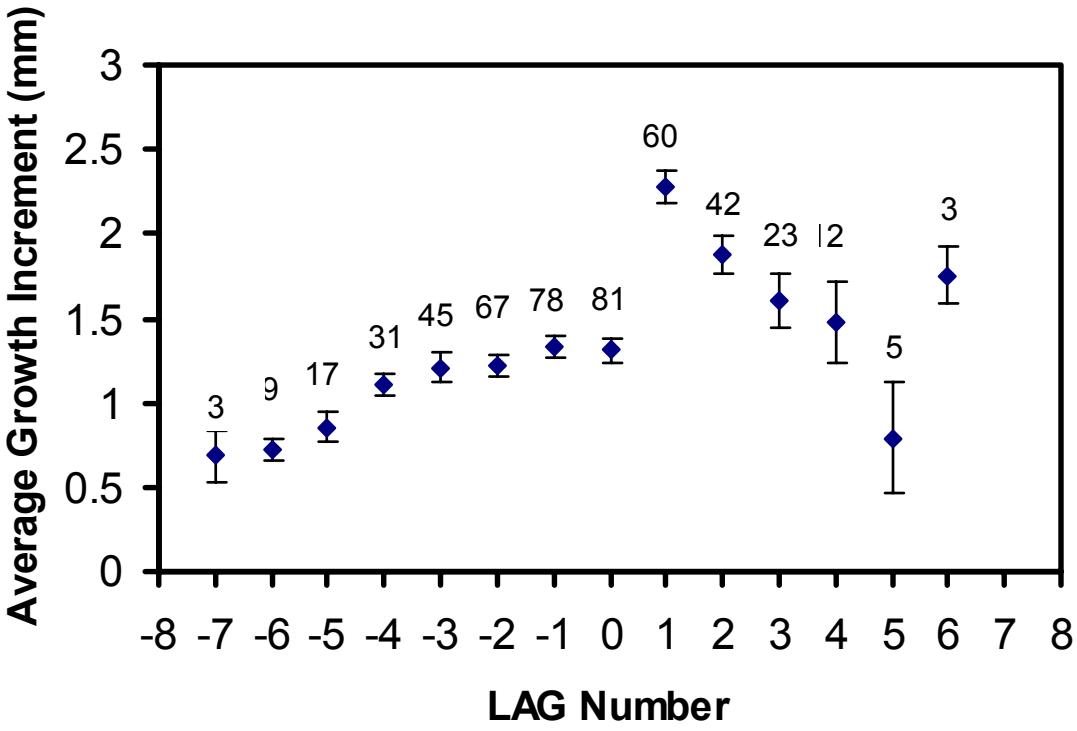


Figure 3.3. Average growth increments. Bars indicate standard error. LAG(0) represents settlement to the benthic habitat. LAG(0) growth is significantly lower than LAG(1) ($P<0.005$). Numbers indicate the sample size for the average growth increments.

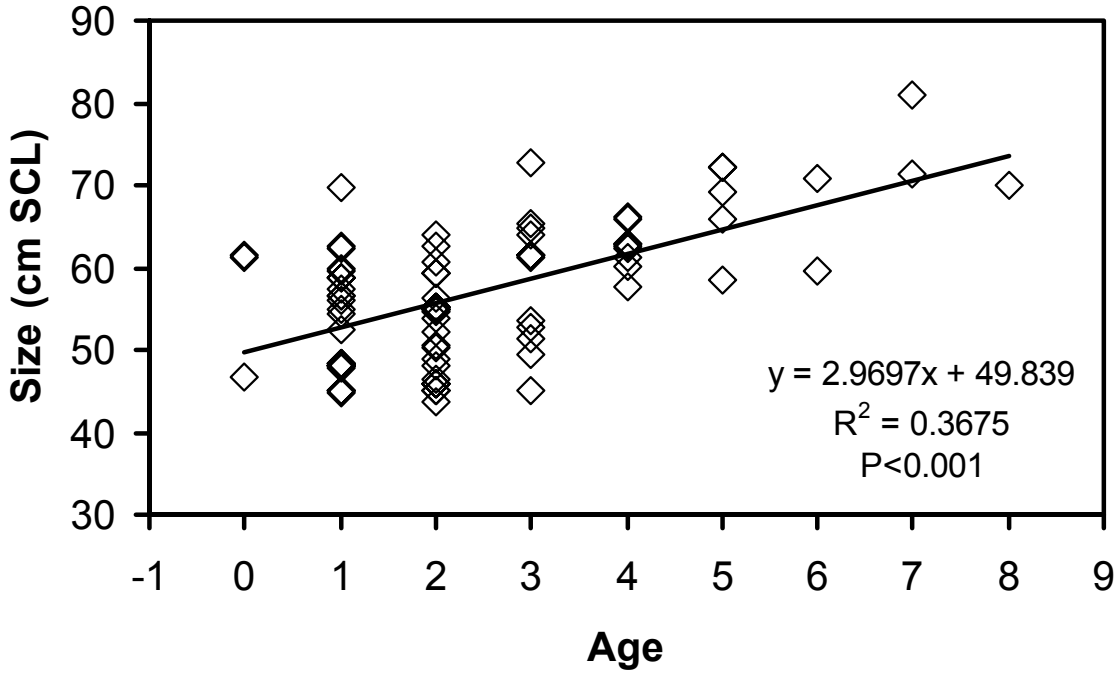


Figure 3.4. Number of LAGs counted after LAG(0) plotted against the animal's carapace length at death (N=84). Solid line represent a least-squares linear regression (P<0.001). At LAG(0) , the linear regression gives a straight carapace length value of 49.8cm SCL.

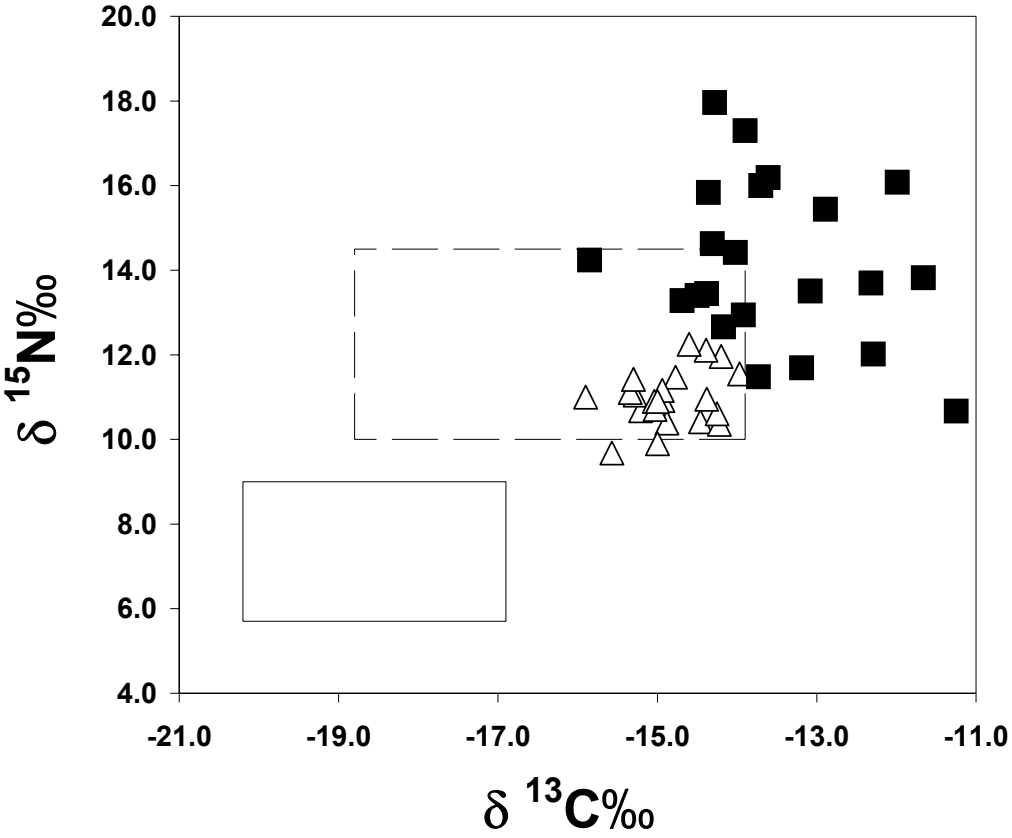


Figure 3.5. Stable isotope ratios from the bones of loggerhead sea turtles. Filled squares were sampled from after LAG(0) and open triangles were sampled between LAG(-1) and LAG(0). Boxes indicate the range of stable isotope values for prey items sampled from the *Sargassum* (solid outline) and from the nearshore benthos (dashed outline, see Table 3.1 species and values).

Table 3.1. Stable isotope ratios for potential prey items. If more than one specimen was analyzed, the sample size is given in parentheses next to the species name, range and mean (mean ± standard deviation) are reported.

Species	$\delta^{15}N$	$\delta^{13}C$
NEARSHORE BENTHOS		
Calinectes sapidus (7)	10 to 12.3 (11.2 ± 0.9)	-17.8 to -13.9 (-16.6 ± 1.5)
Libinia dubia (5)	11.5 to 13.3 (12.6 ± 1.1)	-18.8 to -15.3 (-17.0 ± 1.6)
Limulus polyphemus (9)	11.9 to 14.5 (13.3 ± 0.9)	-17.8 to –16.1 (-17.0 ± 0.6)
Sesarma spp.	10.5	-16.3
Fish from stomach contents	13.7	-16.5
Spartina spp.	3.9	-12.8
Mean of Fauna	**12.4 ± 1.3**	**-16.8 ± 1.1**
PELAGIC SARGASSUM ASSOCIATES		
Lepas spp.	7.6	-20.0
Stomolophus meleagris	8.4	-19.2
Portunus Sayi	7.8	-17.6
Planes minutes	6.3	-17.6
Scyllaea pelagica	6.7	-20.2
Monocanthus hispidus	7.3	-19.3
Leander tenuicorni (2)	8.6, 9	-17.1, –16.9
Leander seucorum	5.7	-17.8
Sargassum spp.	4.3	-17.2
Mean of Fauna	**7.3 ± 1.2**	**-18.1 ± 1.2**

pelagic and benthic fauna, with the values for $\delta^{15}N$ being consistently higher among near shore, benthic feeding animals compared to the animals associated with *Sargassum*. In general the pelagic fauna had more negative $\delta^{13}C$ values than the benthic fauna.

Stable isotope values in bone tissue were compared to values in potential prey items from each habitat (Table 3.1, Fig. 3.5). The average $\delta^{15}N$ value for bone tissue internal to LAG(0) was 11.0 $\pm$ 0.6‰ and for the animals associated with the *Sargassum* the average $\delta^{15}N$ value was 7.3 $\pm$ 1.2‰. The average $\delta^{15}N$ for benthic invertebrates tested was 12.3 $\pm$ 1.3 ‰ and for bone tissue external to LAG(0), the value was 14.1 $\pm$ 1.9 ‰. For the $\delta^{13}C$ values, the *Sargassum* animals had the most negative values (-18.4 $\pm$ 1.3 ‰) followed by the benthic invertebrates (-16.8$\pm$ 1.2 ‰), then the bone tissue interior to LAG(0) (-14.8$\pm$ 0.5 ‰) followed by bone external to LAG(0) (-13.4$\pm$ 1.2 ‰).

Pelagic Stage Duration and Growth Rates

For bone sections that had at least six measurable pre-settlement LAGs , including LAG(0) (N=31), linear regressions fit the individual growth trajectories well (r^2 = 0.949 to 0.998, $p<0.001$). Bjorndal et al. (in review) estimate small pelagic juvenile growth rates using skeletochronology and find that loggerheads are approximately 15 cm SCL at one year of age. In addition, growth rates appear to be linear after age one in small loggerheads (Bjorndal et al. in review).

From the regression equation for bone section width vs. carapace length (Table 2.5 in Chapter 2), 15 cm SCL equates to 4 mm bone width. I back-calculated the regressions for the individual growth trajectories to 4 mm and added one year to estimate age at settlement. Average age-at-settlement was 14.8 ± 3.3 years (mean ± standard deviation). The range of ages-at-settlement was 9 to 24 years. For the pelagic stage, the measured

growth intervals averaged 1.22 ± 0.04 mm bone/year in growth which translated to approximately 2.90 cm SCL/year (Fig. 3.3). For the benthic stage, average of the growth increments for LAG(1) and up (not including the pulse in growth that occurs following LAG(0)) was 1.67 ± 0.08 (SE) mm bone/year, which converted to 3.97 cm SCL/year.

DISCUSSION

I identified settlement marks from bone cross-sections of benthic juvenile loggerheads that use summer foraging habitats along the mid-Atlantic coast of the U.S. The settlement marks indicate the timing of the ontogenetic shift from pelagic to benthic habitats for each individual turtle. This shift resulted in increased individual growth rates consistent with the theory that a primary motivator for an organism to undergo an ontogenetic habitat shift is to maximize its growth rate (Werner and Gilliam 1984). Organisms trade-off high growth rates for decreased risks of mortality from predation early in their development, as documented for bluegill sunfish (*Lepomis macrochirus*) and Nassau grouper (*Epinephelus striatus*) (Werner et al. 1983, Werner and Hall 1988, Dahlgren and Eggleston 2000). These sub-optimal habitats limit growth and eventually an animal must shift to a more profitable habitat to reach reproductive size (Werner and Gilliam 1984).

Stable isotope ratios indicate assimilation of food resources with fractionation occurring as a function of trophic level with $\delta^{15}N$ fractionating 3-4‰ per trophic level (Michener and Schell 1994). Taking this fractionation into account, our $\delta^{15}N$ values were consistent with loggerheads feeding pelagically prior to LAG(0) and in the benthos after LAG(0). Values of $\delta^{13}C$ typically fractionate about 1‰ per trophic level (Michener and Schell 1994). However, fractionation levels between tissues vary (Tieszen et al. 1983,

Hobson et al. 1996). Diet-to-collagen $\delta^{13}C$ fractionation is typically about +3.0‰ (Koch et al. 1994), the fractionation we would expect in comparisons of the muscle from prey to the collagen from sea turtle bones. Thus, our $\delta^{13}C$ values also indicated that animals were feeding on pelagic fauna prior to LAG (0) and on benthic fauna after LAG (0).

The results of the stable isotope analysis strongly supported the hypothesis that the growth rate shift observed in bone cross-sections occurs simultaneously with a shift in diet, representing an ontogenetic habitat shift from the pelagia to the benthos. In other studies of higher marine vertebrates, stable isotope ratios successfully determine trophic relationships (Hobson 1993), temporal dietary records from tooth annuli (Hobson and Sease 1998), diet and trophic level feeding (Godley et al. 1998, Walker and Macko 1999), foraging location and migration patterns (Burton and Koch 1999), and stock structure (Walker et al. 1999).

I estimated size at settlement at 48.5 to 51.1 cm SCL. From a length frequency analysis, average size at settlement for the same population of loggerheads is estimated at 49 cm SCL (Bjorndal et al. 2000b). The agreement between the two values further supports that the settlement mark has been correctly identified in this population of loggerhead sea turtles and that the mark can be used to estimate age based on time since settlement.

The estimation of an average 14-year pelagic juvenile stage is much longer than previous estimates. Bjorndal et al. (2000b) estimate the same value at an average of 8 years to 49 cm SCL. Hatchlings average 4.5 cm SCL, for a loggerhead to get from 4.5 cm to 49 cm in 8 years would require growth rates of 5.56 cm/year. Such high growth rates are improbable for loggerheads in the pelagic environment. This proposed growth rate is

actually higher than Kemp's ridley growth rates for similar sized animals feeding in the benthos (Chapter 4).

The demographic implications of a much longer pelagic stage are dramatic. Previous population models have used six to eight years for the duration of the pelagic stage (Crouse et al 1987, Crowder et al. 1994, Heppell et al 2002). The elasticity of a stage is directly proportional to the length of that stage. Stages with the highest elasticity have the greatest impact on population growth rates with proportional changes in annual survival rates. Of the juvenile stages considered in loggerhead population modeling efforts, benthic juveniles usually have the highest elasticity, but it may be that the pelagic stage is a much more critical developmental habitat than has previously been thought, potentially encompassing half of the total time to reproductive maturity.

The timing of ontogenetic shifts is critical to population dynamics as together they determine life-cycle-stage durations and time to reproductive maturity, as well as influence size-specific growth and survivorship (Werner 1988). Population models developed for loggerheads from the southeast U.S. use fixed stage durations for each of the major juvenile life stages, pelagic and benthic (Crouse et al. 1987, Crowder et al. 1994, Heppell et al. 2002). Sources of mortality within the two habitats used by juveniles likely vary, and the time that animals are exposed to both natural and anthropogenic mortality sources typical of the two habitats is important to understand. Data from individual turtles will provide the basis for more detailed stochastic or individual-based population models (DeAngelis and Rose 1992).

CHAPTER 4

GROWTH AND ONTOGENY IN JUVENILE KEMP'S RIDLEY SEA TURTLES (*LEPIDOCHELYS KEMPI*)

INTRODUCTION

Kemp's ridley (*Lepidochelys kempi*) sea turtles primarily nest at Rancho Nuevo on Mexico's Gulf of Mexico coast, with minor nesting noted along the Gulf coast states of the U.S. and Mexico (TEWG 1998, 2000). Due to their limited distribution, low population size, and previously declining stocks, they are considered one of the most critically endangered sea turtles in the world (2000 International Union for Conservation of Nature and Natural Resources (IUCN) Red List of threatened species, US Endangered Species Act). In recent years, nest numbers at Rancho Nuevo have increased to levels equivalent to the early 1960's, but this level is still far below historical records of Kemp's nesting and conservation efforts need to continue (TEWG 2000, Heppell et al. 2002b).

Kemp's are believed to follow the currents of the Gulf of Mexico and either stay entrained within the Gulf or enter the Loop current, the Florida current and eventually enter the Gulf Stream and migrate up the Atlantic coast of the U.S (Collard 1990, Collard and Ogren 1990). Eventually, juveniles that migrate up the Atlantic coast must recruit back to the Gulf of Mexico as there is no nesting in along the Atlantic coast. This is likely not a sharp transition as the average size of Kemp's appears to increase gradually from north to south along the U.S. Atlantic coast (Carr 1980, Henwood and Ogren 1987), however it is not known if the larger animals in the south are, in fact, older or if they are experiencing higher growth rates. The difference in habitat utilization of juvenile Kemp's has important implications for population dynamics, as animals within the same age classes may be experiencing a range of growth and mortality rates (Gilliam and Fraser 1988).

Heppell et al. (2002b) present a series of models for Kemp's, varying model parameters and age to maturation. They use a four-stage matrix model that includes two juvenile stages, small and large benthic immatures, with the division occurring at six years of age based on the results of a catch-curve analysis. The different habitats used by Kemp's and subsequent variability in growth and mortality rates need to be incorporated into future modeling efforts, therefore there is a need to increase our understanding of Kemp's ontogeny to improve population models and subsequent management decisions (Heppell et al. 2002b).

I demonstrated a high correlation between morphometric measurements of the humerus and carapace length in Kemp's ridleys (Chapter 2). This relationship can be used to infer size-at-age from the diameters of annual lines of arrested growth (LAGs) to develop a large dataset for the determination of size-at-age and growth rates in juvenile male and female Kemp's ridleys. Such a dataset can also provide insights into the variance in body size-at-age caused by changes in the resource utilization as turtles grow, leading to ontogenetic shifts in habitat, diet, and/or growth rates (Lomnicki 1988). Examinations of variance in size-at-age and growth-at-age can give indications of habitat quality and ontongeny (Atchley 1984, Lomnicki 1988, Lynch 1988, Klingenberg et al. 1996).

As with most species of sea turtles, the life history of Kemp's incorporates a major ontogenetic shift from pelagic to benthic habitat utilization (Musick and Limpus 1997). In small Kemp's, I consistently observed a diffuse annuli which was confirmed to be the first year mark (Chapter 2). Often this mark was not followed by any additional marks in turtles stranding in the nearshore, so I hypothesized that Kemp's make the ontogenetic shift from pelagic to benthic habitats in their second year. To test this hypothesis, I sampled bone

tissue interior to the first year mark and exterior to it for evidence of a diet shift consistent with such a habitat shift (Chapter 3).

The purpose of this chapter is to describe the growth, ontogeny and potential age-at-maturation for male and female Kemp's ridleys using methods established in previous chapters and to compare these results with those of previous studies of age and growth in this species (Cailouett et al. 1995, Schmid and Witzell 1997, Zug et al. 1997, Schmid 1998, TEWG 2000). I use the results from skeletochronology for an analysis of the dynamics of growth in juvenile Kemp's, differences that may exist between the sexes, and inference of age-at-maturity from growth rates fit to von Bertalanffy growth curves. Further, I use stable isotope ratios to identify when Kemp's shift from pelagic to benthic habitats.

MATERIALS AND METHODS

Humeri Preparation

I received left front flippers from dead Kemp's ridley sea turtles stranded along the coasts of North Carolina, Virginia and Maryland. For most of the turtles, straight carapace length (SCL) was recorded, measured as standard straight-line length from the nuchal notch to the posterior end of the posterior marginal. If only curved carapace length (CCL) was recorded, it was converted to SCL using the following regression equation generated from 309 paired measurements for Kemp's between 18.4 and 66.2 cm SCL ($r^2 = 0.991$; $P<0.001$):

$$SCL = 0.957*CCL - 0.696.$$

A subsample of 154 Kemp's between 21.7 and 50.5 cm SCL and for which age could be estimated with a high degree of confidence was used for this study. The sample contained 21 males and 54 females with sex confirmed by visual examination of the

gonads. The remaining 79 specimens in the sample did not have sex identified. An additional 13 samples between 50.6 and 62.0 cm SCL were used to estimate growth rates in large individuals for completion of the growth curve. These animals were recovered from Texas. Due to resorption, accurate ages could not be assigned to these animals. For each specimen, I dissected out the humerus then cleaned off the soft tissue by flensing and boiling. A whole 2-3mm thick cross-section was taken just distal to the deltopectoral ridge from each of the dried bones (Chapter 2). These were preserved in formalin for analyses of growth marks. For 10 of the Kemp's specimens, an additional 1mm thick cross-section was taken and saved for stable isotope ratio analysis.

Growth Mark Analysis

The 2-3mm thick sections of bone were decalcified in acid using a commercial preparation, RDO (Apex Engineering Products Corp.,Plainfield, IL, USA). Once decalcified, they were thin-sectioned to 25 μm using a freezing-stage microtome. Thin-sections were stained using Erlich's Hematoxylin diluted 1:1 with distilled water (Klevezal 1996). In a thin-section of bone there are thin lines that stain dark, called lines of arrested growth (LAGs), alternating with broad lighter stained zones, generally considered to represent the region of active growth (Castanet et al. 1993). Together, one LAG and one broad zone make up a skeletal growth mark and represent one year of growth (Chapter 2). In a cross-section, the interior LAGs represent growth earlier in life while the most external LAG was the most recently deposited. Digital images of the stained sections were taken at 10x magnification. Visible growth marks were counted and measured from these images (Fig. 4.1). The interpretation and validation of the growth marks in this species was presented in Chapter 2.

Size- and Growth-at-Age

An age was assigned to each of the 154 specimens that had a complete record of LAGs. Kemp's ridley nests hatch between late May and mid-July (Marquez 1994). Such a narrow range of hatch dates allowed for partial years to be incorporated in age assignments as follows; animals that died from September to November were assigned ages equal to the LAG count plus a quarter year, from December to February, LAG count plus one half year, March to May, LAG count plus three quarters year and June to August were assigned integer age numbers equal to the number of LAGs.

Every LAG diameter was measured on each of the 154 specimens. Measurements were made of LAG diameters on the lateral axis of the bone, parallel to the dorsal edge. All growth increment measurements from the 154 aged animals were used to observe the mean and variance in age-specific growth rates. The growth increment associated with a LAG is representative of the amount of growth that took place the year preceding when the LAG was deposited. Similar analyses were run on the subsets of the data for which sex was reported. Sample sizes for each age and for each sex varied (Table 4.1).

As discussed in Chapter 2, the first growth mark in Kemp's appeared as a diffuse annulus not always clearly visible. When the bound of the mark could be clearly distinguished, this annulus was measured at the outer most points of the darkly stained area (Fig. 4.1). As this mark is not always clearly visible, I was not able to measure it on every bone.

Table 4.1. Resulting sample sizes for size-at-age, in terms of LAG diameters, and growth increments (LAG(x+1) – LAG(x)).

Age	Size-at-Age			Growth Increment		
	All	Male	Female	All	Male	Female
1	97	14	28	59	13	28
2	115	19	50	65	13	50
3	74	14	29	31	6	29
4	33	6	14	17	4	14
5	17	4	6	7	2	6
6	8	2	3	2	1	3
7	3	1	0	1	1	0
8	2	1	0	0	0	0

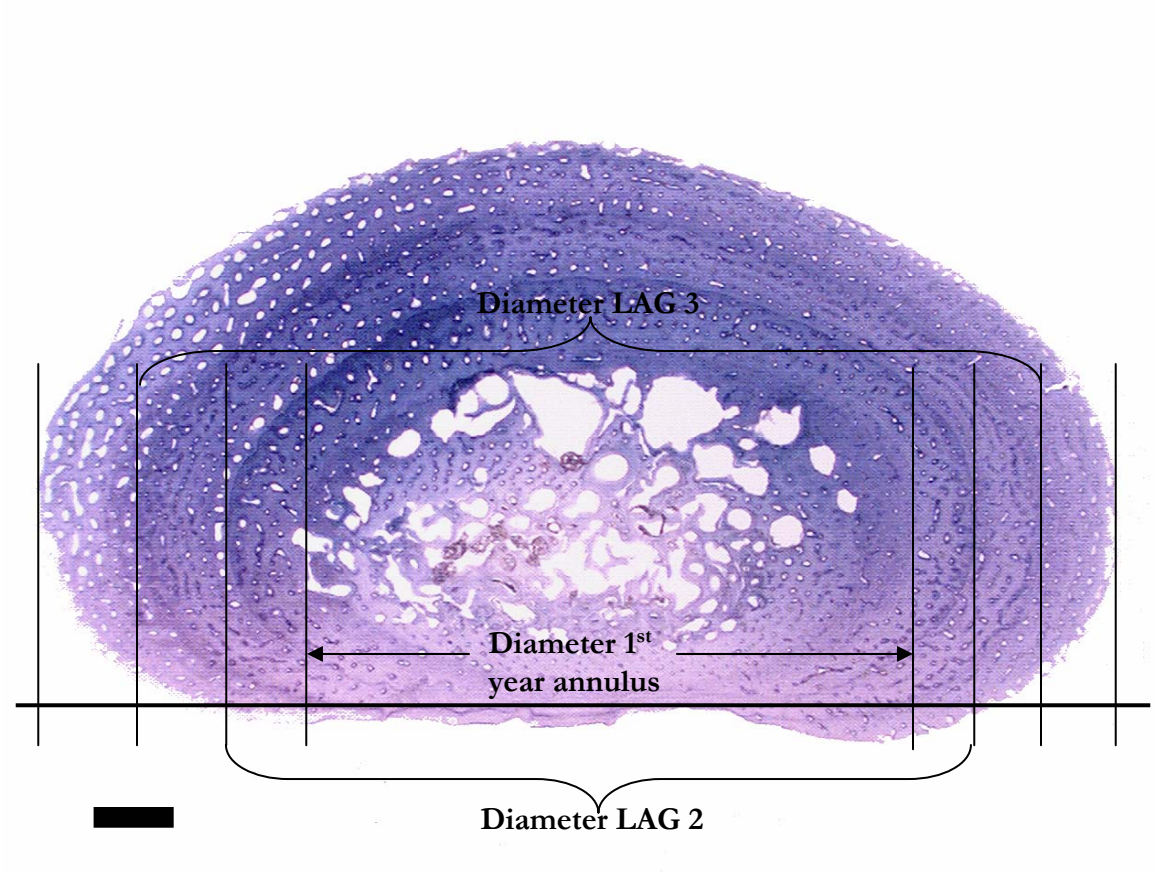


Figure 4.1. Image of humeri cross-sections of a 3.25 year old wild Kemp's ridleys (age estimated from LAG count and stranding date), black bar represent 1mm in length. Diagram demonstrates how LAG and annuli diameters were measured and ages estimated.

For the purposes of doing a nonlinear curve-fit of the von Bertalanffy growth curve, the diameters of the last (outer) two LAGs on each bone cross-section were used, including the 13 for which age could not be assigned. Each diameter measurement was converted to carapace length using the regression equation for carapace length as a function of humerus cross-section width (Table 2.5, Chapter 2). As not every bone had two measurable LAGs, the resulting sample size was 117 one-year growth intervals. The von Bertalanffy growth interval equation was fit to the data from 117 pairs of carapace lengths using the following equation (Fabens 1965):

$$L_1 = L_\infty - (L_\infty - L_2)e^{-k}.$$

In this equation, L_1 and L_2 represent the carapace length estimated from the two outermost LAG diameters with L_1 being the last LAG and L_2 the second to last LAG. Asymptotic length and intrinsic rate of growth are represented by L_∞ and k, respectively. Separate von Bertalanffy curves were fit to the female (N=37) and male (N=43) subsets. Once the L_∞ and k parameters were solved for by the curve-fit, the curves were plotted with size as a function of age using the following equation:

$$L_X = L_\infty - (L_\infty - L_0)e^{-kX}.$$

In this equation, x is age in years and L_0 is initial size.

Stable isotope ratio analysis

The unpreserved bone sections were sampled interior to the first year mark and exterior to it, between the first year mark and the second year mark if there was one, for 10 of the bones (Fig. 4.1). Bone sections were refluxed in distilled dichloromethane prior to isotope analysis to remove lipids. The lipid-extracted bones were sampled using a dentist

drill and the resulting powder was weighed. Samples were analyzed for stable isotopes of ^{13}C and ^{15}N in the same manner as loggerhead bones in Chapter 3.

RESULTS

Size-at-age for juvenile Kemp's ridleys

Using the average size of Kemp's at hatching (van Buskirk and Crowder 1994), growth from age zero to age one appeared to be a separate phase from growth after age one (Fig. 4.2). A curve such as the von Bertalanffy curve that models growth rates as declining linearly from birth to adult will not describe such a shift in growth rates. Therefore, I set L_0 (or more accurately, L_1) in equation (2) at 22cm SCL which is the average size at age one (Table 4.2). The resulting curves from the growth increment data coincided well with the estimated size-at-age data; however, the curve appeared to underestimate growth in the first few years (Fig. 4.2). The intrinsic rate of growth (k) calculated from all of the data was 0.1239, while for females it was 0.1174 and for males, 0.1042 (Fig. 4.3). Size of adult Kemp's ridleys is defined as >60 cm SCL (TEWG 2000). When all of the data were considered, this size was reached at age 12. From the curve fit to females, 60 cm SCL was also reached at age 12 while the age increased to age 15 for males.

Variance in size-at-age and growth-at-age

There was a sharp increase in the variance of the log(LAG diameters) between ages two and four, followed by a sharp decline from age four to age five (Fig. 4.4). The average yearly growth increments peaked at age 5 (Fig. 4.5) and there appeared to be a dip or an

Table 4.2. Mean size-at-age estimations based on LAG counts (Age columns) and von Bertalanffy curve fits (VB columns). In the Age columns, data are reported as mean with standard deviation in parentheses, sample sizes are reported in the N columns. All sizes are in cm SCL. See text for explanation of age assignments based on LAG counts, bold ages are the ages represented by the VB estimates.

	All Samples				Males				Females			
Age (yrs.)	N	Age	LAG	VB	N	Age	LAG	VB	N	Age	LAG	VB
0-**1.0**	11	23.5 (2.08)	21.6(1.		0/14		21.6(1.6)		1/28	24.3	21.5 (1.7)	
1.25-**2.0**	31	27.6 (2.08)		29.7	3/19	27.8 (2.20)	27.3(2.5)	28.6	5/51	28.9 (2.21)	27.4 (2.0)	29.6
2.25-**3.0**	36	35.4 (3.08)		34.7	6/12	34.4 (3.49)	30.4(4.1)	32.8	19/30	35.7 (2.52)	31.2 (3.4)	34.6
3.25-**4.0**	35	36.5 (3.74		39.2	10/4	36.3 (3.59)	30.8(2.5)	36.6	16/14	37.8 (2.86)	34.9 (5.4)	39.0
4.25-**5.0**	14	41.5 (5.23)		43.1	3/4	38.4 (2.55)	35.4(2.5)	40.0	7/6	41.0 (6.40)	36.1 (3.9)	42.9
5.25-**6.0**	10	42.3 (4.91)		46.6	1/ 2	36.2	41.2(3.2)	43.0	4/2	40.8 (6.07)	40.9 (6.4)	46.5
6.25-**7.0**	3	48.0 (1.62)		49.7	1/1	49.8	43.2	45.8	1/ 2	46.7	41.7 (1.8)	49.6
7.25-**8.0**	1	43.1		52.4	0/1		47.2	48.3	1/0	43.1		52.3
8.25-**9.0**	1	50.5		54.8	1/0	50.5		50.5				54.8
10.0				56.9				52.5				57.0
11.0				58.8				54.3				58.9
12.0				60.4				56.1				60.7
13.0				61.9				57.4				62.2

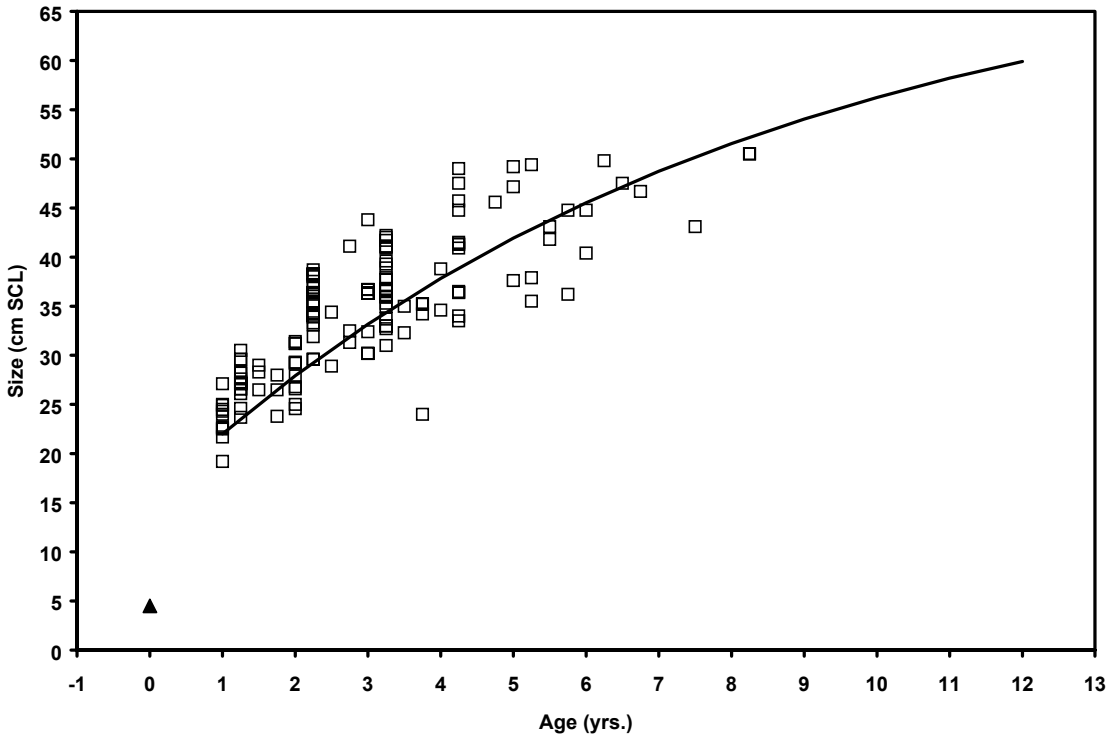


Figure 4.2. Size-at-age for the 154 specimens for which age could be estimated (open squares). Filled triangle represents average hatchling size, considered age 0. Filled circles are the five known-age, wild, coded wire tagged animals that stranded along the Atlantic coast (see Chapter 2). Solid line and equation represent the von Bertalanffy curve that was fit to growth increment data fit to animals up to 60 cm SCL ($R^2 = 0.95$; $P<0.005$). It is shown here overlaid with the size-at-age and known-age data to demonstrate how well growth rates estimated from growth mark diameters describe somatic growth rates.

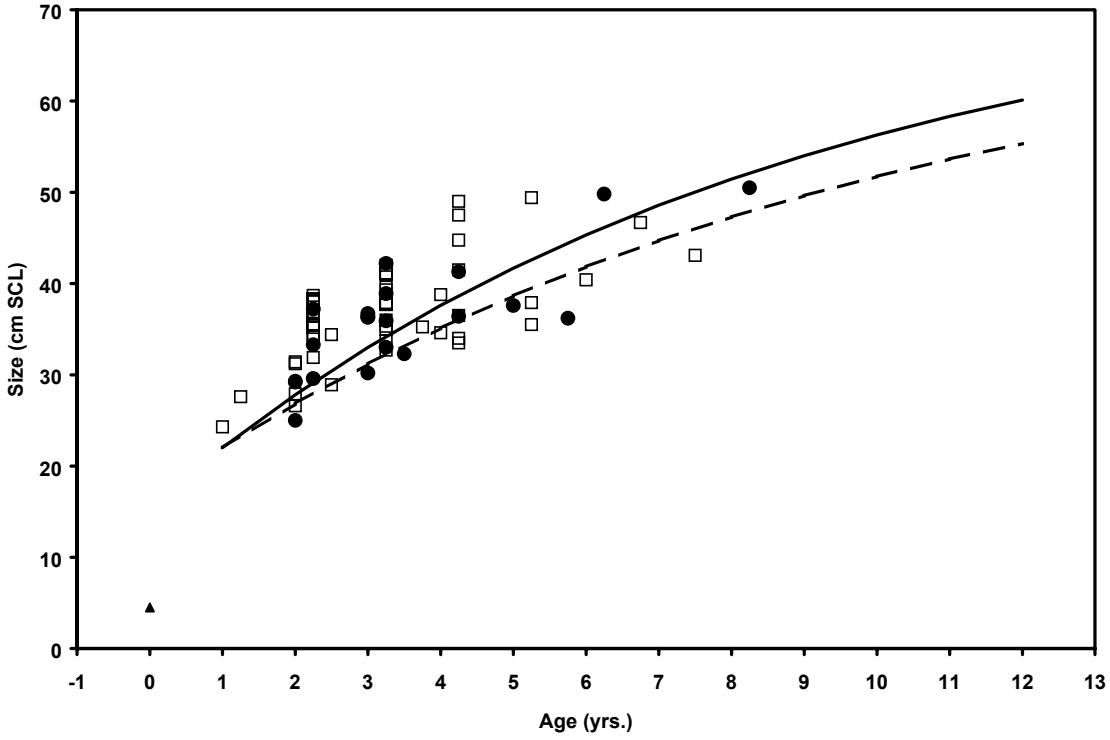


Figure 4.3. Size-at-age for the 21 aged specimens identified as males (solid triangles) and the aged 54 specimens identified as female (open squares). Filled triangle represents average hatchling size, considered age 0. Lines and equations represent the von Bertalanffy curve fit to the growth increment data (dashed line = males, $R^2 = 0.95$, $P<0.005$; solid line = females, $R^2 = 0.90$, $P<0.005$).

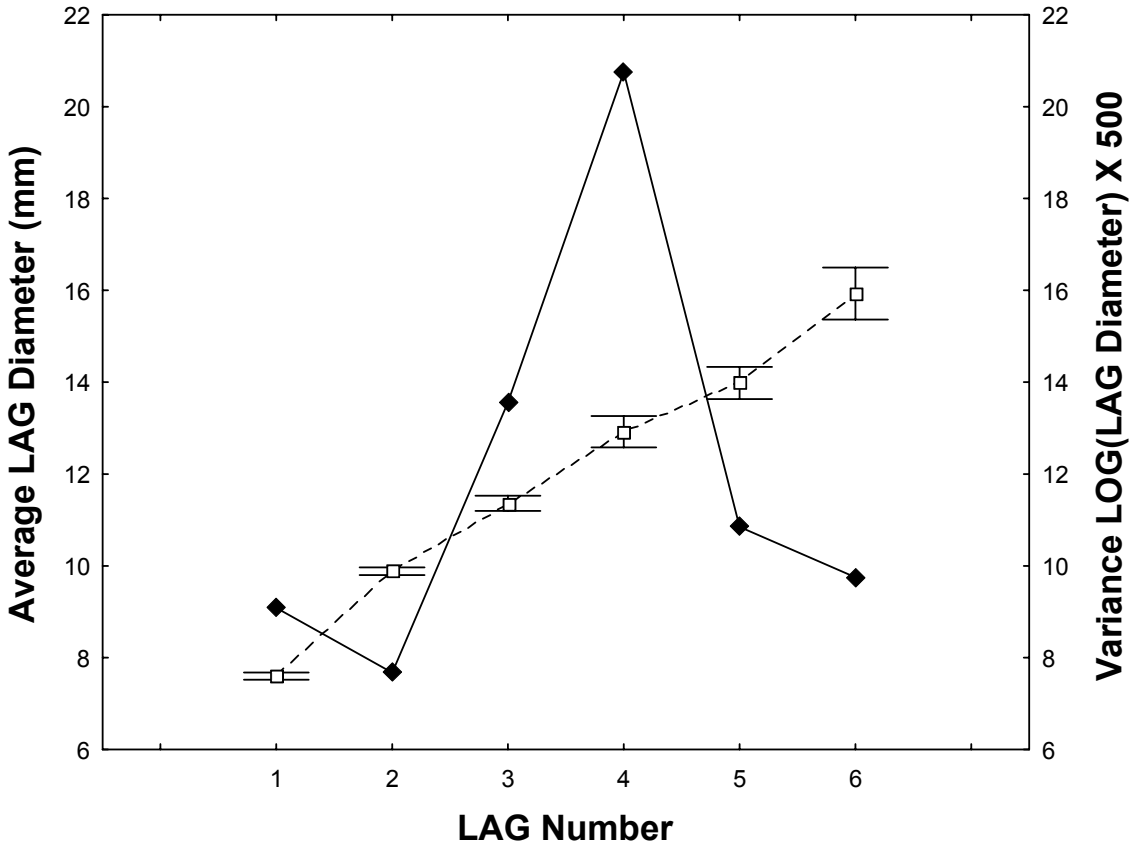


Figure 4.4. Average LAG diameters (dashed line) and the variance in the log of the LAG diameters (solid line) for all of the data (combining sexes). See Table 4.1 for sample sizes. Bars indicate standard errors.

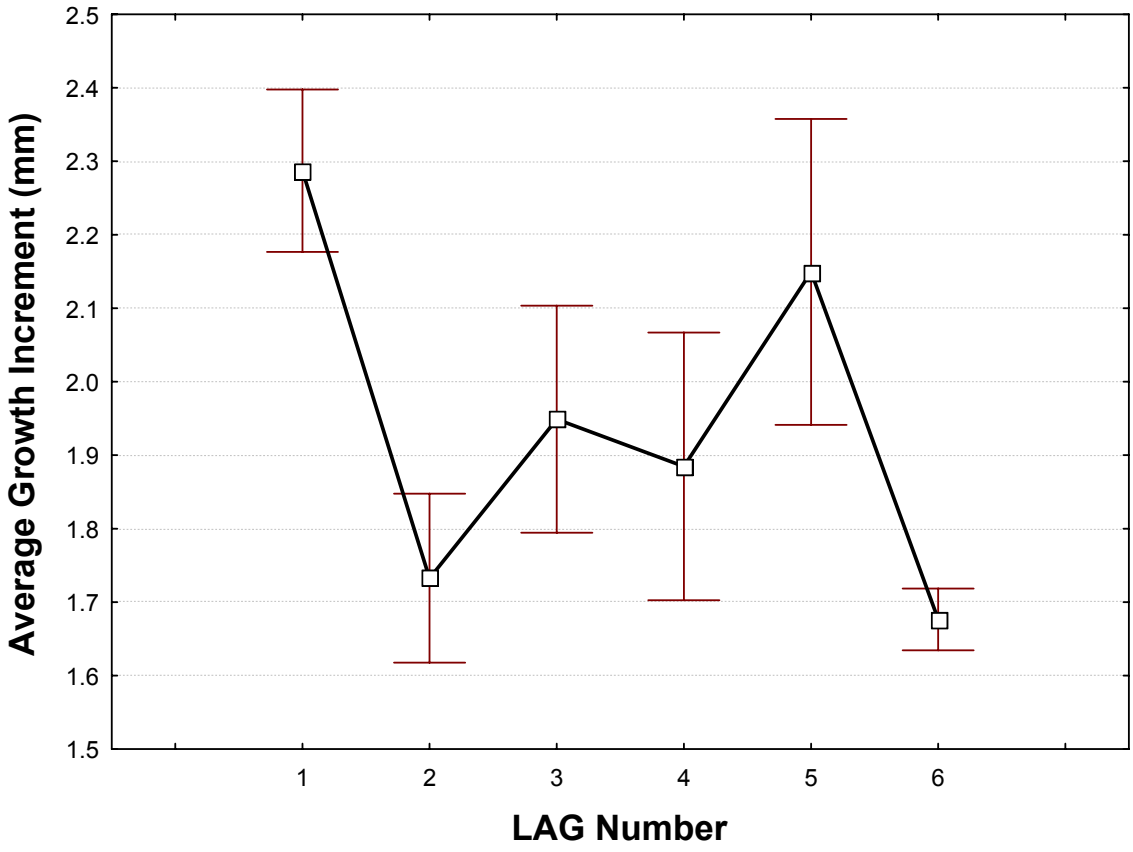


Figure 4.5. Average growth increments as measured by the difference between successive LAG diameters. See Table 4.1 for sample sizes. Bars represent standard error.

inflection point between ages four and six in the growth curve of LAG diameter when only average diameter was observed (Fig. 4.4).

When sex was taken into account, similar peaks were still observed in the variance of the log(LAG diameters) for both sexes, but, the spike in variance occurred one year earlier for male Kemp's than for females (Fig. 4.6). Similarly, the dip in average LAG diameters occurred between ages three and five in males and ages four and six in females (Fig. 4.7). The greatest difference in size-at-age between males and females occurred at age four, after this age, female growth rates decreased and male growth rates increased, resulting in similar size-at-age (Fig. 4.7&4.8). Average growth increments were low from ages two to three in males and from ages two to four in females (Fig. 4.8). At age four in males and age five in females, average growth increments increased.

Stable Isotope Ratios

Based on two-sample T-tests with unequal variances, the average stable isotope ratios for ^{15}N were significantly higher for within the first growth mark versus bone sampled external to it ($P<0.005$, Fig. 4.9). The difference between the means was 4.64 ‰. The $\delta^{13}C$ values were significantly more negative after the first growth mark than within it, the absolute difference between the averages was very small at 0.84 ‰ ($P=0.007$; Fig. 4.9).

DISCUSSION

Kemp's ridleys underwent a shift in diet after age one, from lower to higher trophic levels. The change in the average $\delta^{15}N$ values between the first and second year marks is consistent with a shift from feeding on epi-pelagic invertebrates to feeding on nearshore

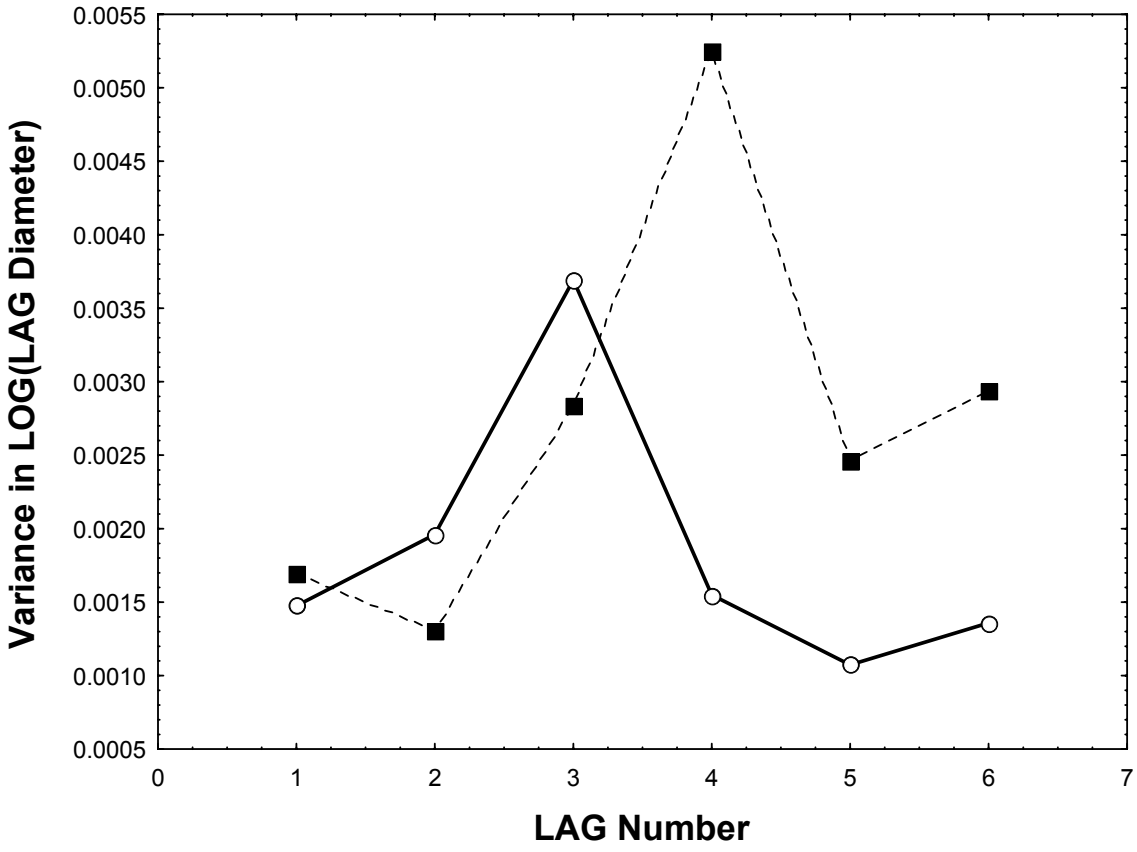


Figure 4.6. Variance in the $\log_{10}$ of the LAG diameters for males (solid line with open symbols) and females (dashed line with filled symbols). See Table 4.1 for sample sizes.

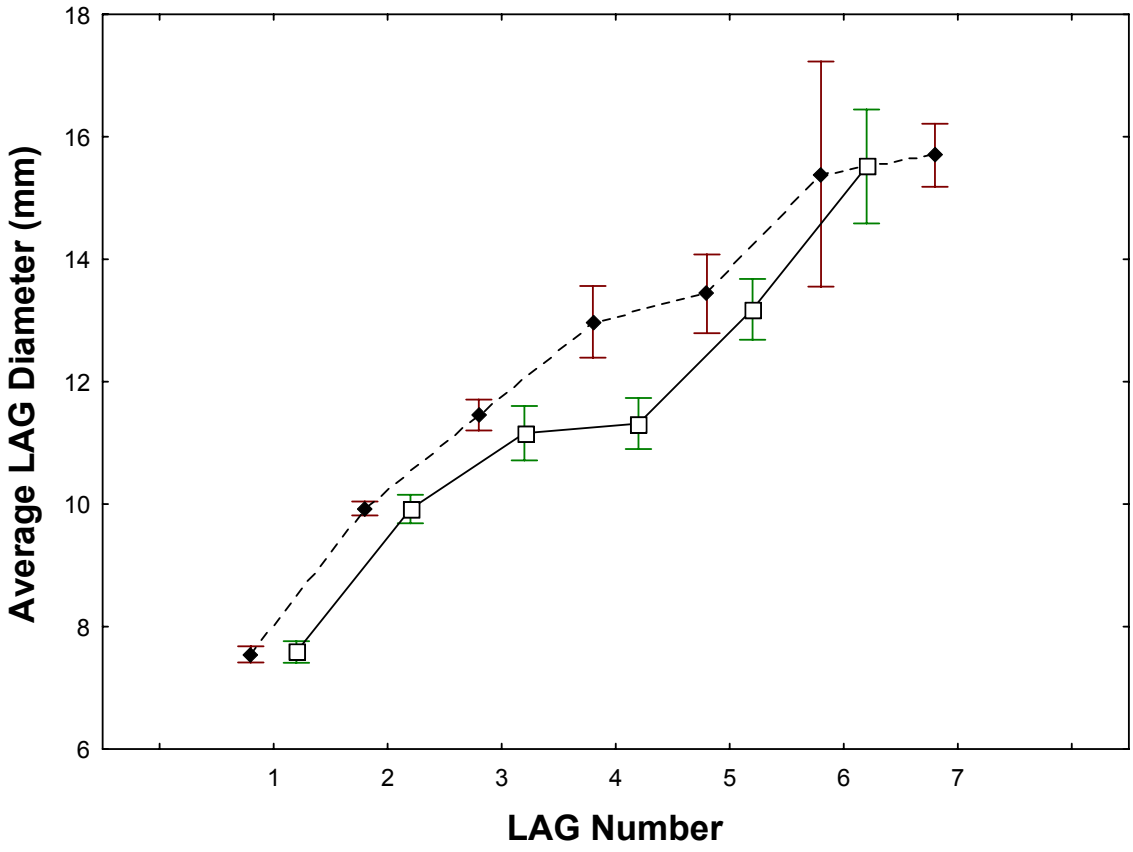


Figure 4.7. Average LAG diameters for males (solid line with open square symbols) and females (dashed line with filled diamond symbols). Bars indicate standard errors.

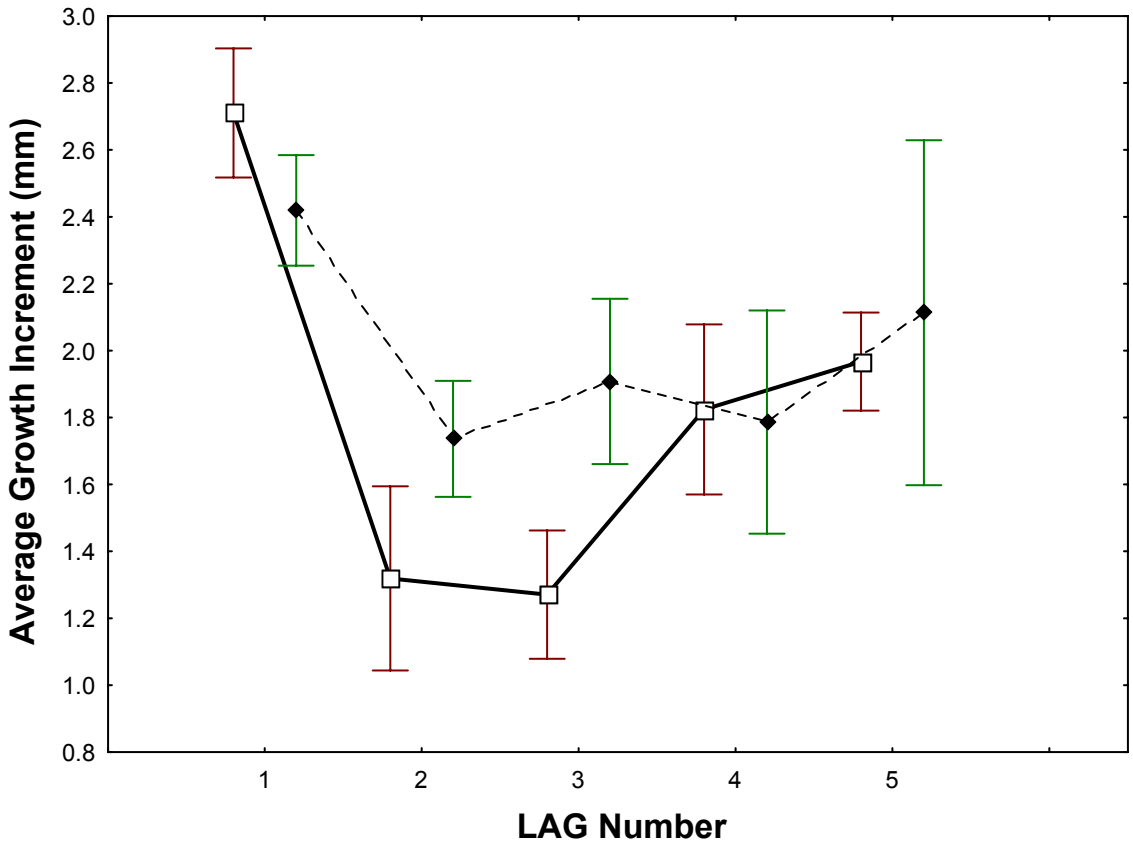


Figure 4.8. Average growth increments as measured by the difference between successive LAG diameters. Males are represented by the solid line and open square symbols, females by the dashed line and filled diamonds. Bars indicate standard errors.

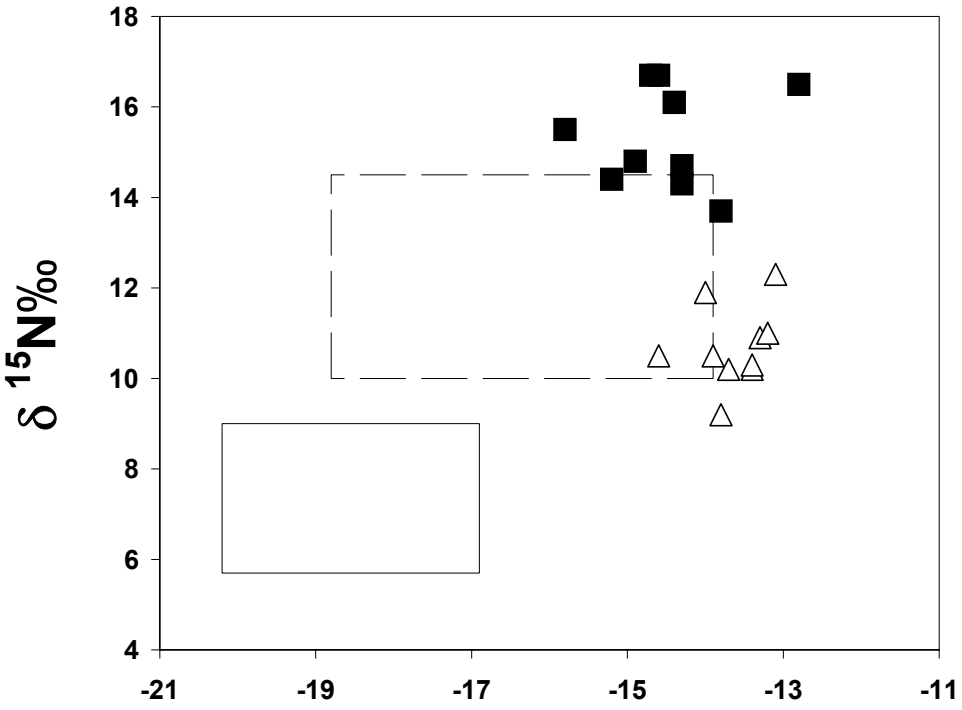


Figure 4.9. Stable isotope ratios from the bones of Kemp's ridley sea turtles. Open triangles were sampled from inside the first growth mark, filled squares were sampled from within the second growth mark. Boxes indicate the range of stable isotope values for prey items (see Table 3.1).

invertebrates (Table 3.1 in Chapter 3; Fig. 4.9). From this it appeared that Kemp's following a post-hatching migration out of the Gulf of Mexico and into the Gulf Stream are pelagic only during their first year of life. The pattern of $\delta^{13}C$ was difficult to interpret as values should become less negative as animals feed closer to shore. Primary summer foraging habitats for small Kemp's are along the northern US Atlantic coast, from New York to Massachusetts (Lazell 1980) and we did not sample near shore prey items from this region, which may explain the difference in $\delta^{13}C$ patterns between loggerheads and Kemp's (Chapter 3).

Inflection points in individual growth curves are usually related to ontogentic shifts, either behavioral or physiological, (Von Bertalanffy 1960, Eisen 1975, Atchley 1984). Surges in growth rates often coincide with ontogentic shifts to more profitable habitats (Werner and Gilliam 1984, Gilliam and Fraser 1988) and a decline in the profitability of a habitat for a given size-class results in increased variance in size-at-age and growth rates (Lomnicki 1988). Chaloupka and Zug (1997) detect a polyphasic inflection point in Kemp's ridley size-at-age data in turtles in a size range that was consistent with the inflection points I observed in the size-at-age data. The combined evidence of the inflection points, and peaks in variance in size-at-age followed one to two years later by increases in growth rates indicated that Kemp's were undergoing shift in growth rates, beginning at age three in males and age four in females.

Based on my stable isotope findings, this shift did not appear to be the shift from pelagic to benthic habitats. Also, Kemp's at sizes smaller than these shifts have been observed with benthic organisms in their digestive tracts. In the waters adjacent to Long Island, NY, USA, Burke et al. (1993) found that 90% of the diet for Kemp's ridley (27.4 – 37.8 cm SCL) were composed crabs, with the predominant species being the nine-spined

spider crab, *Libinia emarginata*, and the Atlantic rock crab, *Cancer irroratus*. In Georgia, USA, common prey species for Kemp's ridleys (29.2 – 32.5 cm CCL) are blue crabs (*Callinectes sapidus*), spider crabs (*Libinia dubia*), stone crabs (*Mennipe mercenaria*), and mud snails (*Nassarius obsoletta*) (Frick and Mason 1998). Species composition of the diet does not appear to vary as a function of size as Kemp's from 40.0 – 69.0 cm CCL have similar diets (Frick and Mason 1998) although it is not known if the size of prey taken increases with size. The observed growth rate shift could reflect a shift to a new resource, such as larger prey.

The change in growth rates could also be indicative of an ontogentic habitat shift not recognized before. The smallest size classes of Kemp's along the Atlantic are found in New England waters (Carr 1980). Assuming that this is where the animals were feeding when the second growth mark was deposited, it is not certain what their diets consist of though it appears to be somewhat different from loggerheads in the mid-Atlantic states based on stable isotope analysis (Chapter 3, current study). Starting at size-classes in the upper 20 cm SCL range (between ages two and four from results presented here), Kemp's are noted further south with benthic crab species similar to those of loggerheads in their digestive tracts (Burke 1993, Frick and Mason 1989).

The shift detected from the analyses of the growth rates may mark the transition from a secondary, post-pelagic small juvenile stage to a larger benthic juvenile stage. Why might small, post-pelagic juveniles opt to settle along the North Atlantic seaboards, if growth rates there are lower? Ontogenetic niche shift theory predicts that at very small sizes, Kemp's are likely more vulnerable to predation, and, potentially, the use of the New England habitat, while resulting in lower growth rates, may have an advantage in reduced mortality rates, providing a growth versus mortality trade-off (Werner and Gilliam 1984, Werner 1988). Shifts to more profitable habitats are often accompanied by surges in growth rates

(Gilliam and Fraser 1988, Chapter 3), such as what was observed in the growth rates presented here between ages four and five. The sex-specific behavior in the timing of the shift is interesting and this is the first time such a difference between the sexes has been noted in a species of juvenile sea turtle. This could have implications for sex-specific growth and mortality rates in small juveniles.

Further studies are needed to determine if this growth rate shift is indeed indicative of an additional life stage in the Kemp's ridley sea turtle and if a similar shift occurs in turtles that remain in the Gulf of Mexico. This information is critical to our understanding of the effects of fishery bycatch on different stages as well as population growth and recovery.

The von Bertalanffy growth curve models growth rates as monotonically declining from birth to adulthood. Age-specific growth rates in Kemp's, however, did not follow this pattern. Neither did they display the parabolic nature assumed by growth functions that incorporate inflection points such as the logistic and Gompertz curves. Growth rates were initially high, then declined for two to three years, depending on sex, then increased again. This is contradictory to the previous findings from a mark-recapture study of steadily declining growth rates (TEWG 2000). The nature of the age-specific growth rates I found demonstrated a polyphasic nature in juvenile growth rates as indicated by Chaloupka and Zug (1997).

Fits of von Bertalanffy curves are still important as they model average growth and allow for comparisons among studies using both similar and dissimilar methods. Other studies have estimated the intrinsic rate of increase (k) at 0.1665 to 0.5774 for Kemp's from the Atlantic coast (Schmid and Witzell 1997, Zug et al. 1997, TEWG 2000) and 0.0852 to 0.317 for animals from the Gulf of Mexico (Table 4.3; Caillouet et al. 1997, Schmid and Witzell 1997, Schmid 1998, TEWG 2000). Schmid (1998) reports annual growth rates for

Kemp's at a mean of 4.6cm/yr. for animals in the 30-40 cm SCL size, 6.2cm/yr. for animal 40-50 cm SCL and 4.6cm/yr. for animals 50-60 cm SCL. The peak in growth rates from 40-50 cm SCL is consistent with the peaks observed in this study which occur in females after age five years and males after age four.

Literature estimates of age-at-maturation range from 10 to 16 years (Table 4.3; Caillouet et al. 1997, Schmid and Witzell 1997, Zug 1997, TEWG 2000). My estimate of 12 years to maturation for females was consistent with this range. The von Bertalanffy curve indicated slower growth and lower asymptotic size for males than for females, resulting in an estimate of 15 years-to-maturity for males. This value is within the range of those reported by other investigators, however, as it is not known at what size males typically mature and the sample size of males was small, this value should be viewed with caution. When compared with size-at-age data, the von Bertalanffy curves appeared to underestimate size-at-age in the early portion of the curve, which would alter the estimation of time to reproductive maturation.

Heppell et al. (2002b) developed a series of population models for the Kemp's ridley and found that the model that best fit the empirical data incorporated an age at maturation of 10 years. Kemp's are believed to follow the currents of the Gulf of Mexico and either stay entrained within the Gulf or eventually enter the Gulf Stream and migrate up the Atlantic coast of the U.S (Collard 1990, Collard and Ogren 1990). As noted previously, habitats as different as the Gulf of Mexico and the mid- and northern Atlantic coast likely

Table 4.3. Summary of von Bertalanffy growth curve parameters estimated from other studies.

Region	Size at Maturation	Time to Maturation	Asymptotic length	Growth coefficient k	Method	Reference
GOM*	60.0 cm SCL	10 yrs	62.27 cm SCL	0.317	M-R†	Caillouet et al. 1995
Atlantic/GOM*	64.2 cm SCL	12-13 yrs	80.0 cm SCL	0.1292	M-R†	Schmid and Witzell 1997
Atlantic/GOM*	65.0 cm SCL	15.7 yrs	79.4 cm SCL	0.130	S‡	Zug et al. 1997
GOM*	-	N.E.§	91.4 cm SCL	0.0852	M-R†	Schmid 1998
Atlantic	64.0 cm SCL	12-13yrs	73.2 cm SCL	0.1665	M-R†	TEWG 2000
GOM*	64.0 cm SCL	10-11 yrs	71.1 cm SCL	0.2095	M-R†	TEWG 2000
Atlantic	60.0 cm SCL	12 yrs	73.0 cm SCL	0.124	S‡	Present Study

* Gulf of Mexico
† Mark-Recapture
‡ Skeletochronology
§ Not Estimated

present different environments for growth and, subsequently, juvenile growth rates between the two habitats are likely very different. This study focused on stranded Kemp's from the mid-Atlantic coast region and the growth rates and potential ages at maturation were representative of small to large juvenile turtles that entered the Gulf Stream. For a more complete understanding of Kemp's life history, a similar analyses needs to be done for juvenile Kemp's that strand in the Gulf. It is not yet known what proportion of Kemp's hatchlings remain in the Gulf of Mexico and what proportion emigrates to the Gulf Stream. Techniques presented here, growth mark and stable isotope analyses, can be applied to this question. Results of this study emphasize the value of skeletochronology, especially when combined with other techniques, for developing a better understanding of the life history the Kemp's ridley and of sea turtles in general.

CHAPTER 5

LIFE CYCLE STAGE DURATION, AGE TO SEXUAL MATURITY AND LONGEVITY IN LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) FROM THE WESTERN NORTH ATLANTIC: CONSEQUENCES FOR CONSERVATION

INTRODUCTION

Specific life histories of individual sea turtle species vary but the common denominator in all of them is that they are long-lived, slow-growing species that use multiple habitats over their course of development. Numerous authors have recently highlighted the management and conservation issues that are critical to maintaining long-lived, slow-growing species (Congdon et al. 1993, Heppell 1998, Crouse 1999, Heppell et al. 1999, Musick 1999). All of these studies emphasize the need for high survival rates in the large juvenile, sub-adult and adult stages to achieve positive or stable long-term population growth. A general conclusion for sea turtles and species with similar life histories is that they are unlikely to be able to sustain even moderate increases in mortality, especially if the populations are already at reduced levels. Sea turtles are subjected to multiple sources of anthropogenic mortality throughout their life cycles. In some regions they are still actively harvested for meat or eggs. For most regions, though, mortality is caused by indirect interactions with fisheries or through the ingestion of marine pollutants (Heppell et al. in press).

The loggerhead (*Caretta caretta)* is distributed globally (van Buskirk and Crowder 1994) and is probably the most well studied of the sea turtles. As with most species of sea turtle, the life cycle is composed of hatchling, pelagic juvenile, benthic juvenile, sub-adult and adult stages (Musick and Limpus 1997). For loggerheads from the western Atlantic,

hatchlings and post-hatchlings are caught up in the Gulf Stream and eventually become entrained in the North Atlantic gyre (Carr 1987, Hays and Marsh 1997, Lohmann et al. 2001). Now juveniles, they remain pelagic, completing a full transatlantic migration before returning to the western North Atlantic (Carr 1987, Bolten et al. 1998). After juvenile loggerheads return to the western North Atlantic, they enter a benthic life stage, inhabiting coastal waters of the mid- and southeast United States for the duration of their life (Carr 1987).

The Turtle Expert Working Group (TEWG 1998, 2000) recognizes four genetically distinct nesting subpopulations in the Western North Atlantic, with an additional fifth added to the list by NMFS-SEFSC (2001). These subpopulations occur as follows: 1) from North Carolina to northeast Florida; 2) South Florida; 3) Florida panhandle; 4) the Dry Tortugas; and 5) the Yucatan Peninsula in Mexico. The subpopulations are defined based on the results of mtDNA analyses and are a consequence of natal nest site fidelity in adult females (Bowen et al. 1992, Encalada et al. 1998, Laurent et al. 1998, Francisco et al. in press). Because of natal nest site fidelity, if a nesting subpopulations is extirpated it is not likely to be recolonized. It is not known if the subpopulations are truly genetically isolated or if there is male mediated gene flow between them. The northern subpopulation, extending from North Carolina to northeast Florida, is of particular concern as it is relatively small, with an estimated 3800 adult females in the population (TEWG 2000, NMFS-SEFSC 2001).

There are not enough long-term data sets to provide unequivocal evidence of continued declines or recovery in the northern subpopulation (TEWG 2000). One exception is a report by Hopkins-Murphy et al. (2001) where aerial surveys for nest

numbers in South Carolina indicate that numbers are declining at a rate of about three percent per year. Other analyses suggest a 0% per year trend, which still means that the northern subpopulation is not recovering (TEWG 2000). This is an alarming possibility for the northern subpopulation of loggerhead sea turtles. The South Florida subpopulation of loggerheads is one of the largest in the world and is therefore critical to the maintenance of this species. Current trends in terms of nest numbers from nesting beaches indicate that this subpopulation is increasing at a rate of about 4% per year but these trends are based on relatively short term data sets (TEWG 2000, NMFS-SEFSC 2001)

As with all species of sea turtle, the sex of loggerhead hatchlings is environmentally determined by a restricted range of nest incubation temperatures. Pivotal and transitional ranges of temperatures determine if the nest will produce males, females or both (Mrosovsky and Pieau 1991). Mrosovsky and Provancha (1989) suggest that most of the eggs produced in a major rookery near Cape Canaveral, Florida incubates at such warm temperatures that virtually no males are produced. Presumably because of a shorter nesting season, characterized by cool beginning and ending temperatures, males are predominately produced in the Northern subpopulation. NMFS-SEFSC (2001) estimated that the northern subpopulation produces 35% female offspring and that the south Florida subpopulation produces 80% female offspring. It is possible that the more northern nesting aggregations in the Southeast U.S. are the primary source of males for the more southern nesting aggregations found in the Western North Atlantic. Thus, extinction of the Northern subpopulation would have negative impacts on the south Florida subpopulation as well.

Crouse *et al.* (1987) present a matrix population model for loggerhead sea turtles from the southeast U.S. Their findings are consistent with what is known about long-lived, late-maturing species. Population growth rates for loggerheads have the highest sensitivity to survival of the juvenile and sub-adult stages, indicating that these life stages are where conservation efforts needed to be increased. The National Research Council (1990) indicates that incidental capture in shrimp trawls was the primary source of anthropogenic mortality of sea turtles. Turtle Excluder Devices (TEDs) are devices that provide an escape hatch in the trawl net to release turtles before they drown. Crouse *et al.* (1987) demonstrated the need for TED regulations. Crowder *et al.* (1994) revisited the model and analyzed the effects of TED regulations on population trends. Their findings show that required use of TEDs in all waters at all times could stabilize or result in increasing population trends if mortality rates for large turtles were reduced by at least 30%.

Since Crowder *et al.* (1994), new information has surfaced regarding the vital rates used in the original model and the effectiveness of TEDs on all benthic life stages. Braun-McNeill *et al.* (in prep) present preliminary growth data from a mark-recapture study indicating that benthic juvenile growth rates in North Carolina estuaries are much slower than those estimated in the original models. In addition, Epperly and Teas (1999) provided evidence that loggerhead turtles larger than 70 cm SCL do not fit through the smallest NMFS-approved TED openings.

Heppell *et al.* (2002a) revised the model, changing the structure somewhat by making it an age-based, pre-breeding census Leslie matrix model that incorporates variable remigration intervals. They looked at the effect of increased stage durations and considered how the results would differ if TEDs only reduced mortality in small benthic juveniles as

compared to all benthic life stages. The results show that with the extended stage durations, reducing mortality in small benthic juveniles is not enough to stabilize population growth rates. TEDs must also convey a reduction in mortality to larger benthic juveniles to result in positive population growth rates. The primary management recommendation from this study is that TED regulations need to be revised to require openings large enough to release adult-sized loggerheads.

Not considered in the previous models is the potential impact of increases in anthropogenic mortality in the pelagic stage, due primarily to the longline fishery in the North Atlantic. The total number of hooks being fished in the North Atlantic has increased by about 50% since 1990 (NMFS-SEFSC 2001). NMFS-SEFSC (2001) revisited the loggerhead matrix model and incorporated perturbations in the pelagic stage survival rates. They found that changes in this stage could have impacts on population growth rates. In response to this, a Biological Opinion was released requiring the closure of the Northeast Distant statistical zone in the North Atlantic to the U.S. longline fleet and implementing take limits in other regions (Anonymous 2001).

For their stock assessment, NMFS-SEFSC (2001) used the values of Bjorndal et al. (2000b) to estimate the length of the pelagic stage at seven to eight years. Similar pelagic juvenile stage durations were used by Crouse et al. (1987), Crowder et al. (1994) and Heppell et al. (2002a). This estimate appears to be low by almost half (Chapter 3) indicating that mortality rates in this stage may be more critical to population growth rates than has previously been indicated (Crowder et al. 1994, NMFS-SEFSC 2001, Heppell et al. 2002a).

I have demonstrated the value of skeletochronology data for the evaluation of growth rates and stage durations in both the loggerhead and Kemp's ridley sea turtles

(Chapters 2-4). Here I apply the same techniques to estimate the duration of the benthic juvenile stage and age-at-maturity for loggerheads. In addition, I estimate post-reproductive longevity from LAG counts at the periphery of the humerus in adults. At the outer edge of the humeri of adult sea turtles, there is a sharp demarcation where LAGs become compressed (Chapter 2). Termed 'rapprochement' by Francillon-Vieillot et al. (1990), similar compression of LAGs has been noted to coincide with sexual maturity in amphibious species (Senning 1940, Kleinenberg and Smirina 1969 and Gibbons and McCarthy 1983, El Mouden et al. 1997). Longevity is estimated by counting the number of lines of arrested growth (LAGs) after rapprochement.

I analyze these data for new estimates of survival rates for benthic juveniles and adults and revisit the current population model (Heppell et al 2002a) to determine the impacts of this new information on stage elasticities and management scenarios.

MATERIALS AND METHODS

Growth mark analysis

The same 84 humeri thin-sections from Chapter 3 were used for benthic growth rate estimates for the purpose of fitting a von Bertalanffy growth curve to describe benthic-stage growth. These were supplemented with an additional 18 humeri ranging from 64.1 to 89.8 cm SCL to add large animals to the sample set for fitting the growth curve. As the purpose of this was to estimate benthic growth rates, animals that didn't have at least one LAG past the settlement mark were not used. The final sample size for the growth curve was 77 loggerhead humeri ranging from 43.6 to 89.8 cm SCL. In adults, LAGs were compacted on the lateral edges of the bone and growth rates could not be estimated. However, the LAGs were still differentiable on the dorsal edge of the bone and there was

an obvious demarcation in adult animals where the compression of LAGs began. I counted the number of LAGs after rapprochement as a proxy measurement for post-reproductive longevity. Sample sizes were 12 males and 20 females that were recovered along the Atlantic coast from Maryland to North Carolina between 1997 and 2000. Diameters of the last (outer) two LAGs on 77 humeri were measured as in Chapter 3. Each diameter measurement was converted to carapace length using the regression equation for carapace length as a function of humerus cross-section width (Table 5, Chapter 2). This resulted in 77 one year growth intervals.

Estimation of benthic juvenile stage duration

The von Bertalanffy growth interval equation was fit to the 77 data pairs of carapace lengths using the following equation (Fabens 1965):

$$L_1 = L_\infty - (L_\infty - L_2)e^{-k}$$

In this equation, L_1 and L_2 represent the carapace length estimated from the two outermost LAG diameters with L_1 being the last LAG and L_2 the second to last LAG. Asymptotic length and intrinsic rate of growth are represented by L_∞ and k, respectively. Once the L_∞ and k parameters were estimated, the curves were plotted with size as a function of age using the following equation:

$$L_X = L_\infty - (L_\infty - L_0)e^{-kX}.$$

In this equation, x is age in years and L_0 is initial size. To observe size-specific growth rates, the one year growth increments were pooled over 10 cm size class ranges based on size at L_1.

Survival Rates

I used strandings data from the Sea Turtle Stranding and Salvage Network to create a catch-curve for animals that stranded dead in 1987 (N=1222; size range of 27 to 100 cm SCL). The von Bertalanffy growth equation (see above) was used to estimate age from size for each of the stranded turtles. The number in each age class was natural log transformed and plotted as a function of age. Linear regressions were fit to the data and instantaneous mortality estimated as the negative of the slope of the regression. This method assumes stable populations and populations were likely in decline prior to 1990, so mortality rates are likely to be underestimated. Therefore, I adjusted the survival rates for a population declining at 3% per year by simulating a population with initial recruitment of 1000 animals per year. Nineteen cohorts were simulated, with age zero recruitment declining at 3% per year. Survival rates from the catch-curve were applied to each cohort to estimate the number remaining after 19 years. At year 19, a cross-section of the simulated population was taken. The cross-section consisted of the age 0 animals from cohort 19, the age 1 animals from cohort 18, etc. The number of animals remaining in each cohort was natural log-transformed and entered into a new catch curve and new survival rates estimated. These survival rates were proportionally lower than the original survival rates. The calculated proportions were applied to the original survival rates, the survival rates recalculated, and the population simulation was run again. The catch curve resulting from the second simulation gave survival rates equivalent to the survival rates from the 1987 strandings catch-curve. Hence, the lower survival rates used to create the second simulated catch-curve are likely more representative of actual survival rates under a 3% per year population decline.

Setting up the Model

I used the same model structure as Heppell et al. (2002a). This is an age-based Leslie matrix model incorporating a pre-breeding census with survival to age one incorporated into the fecundity function (Caswell 2000). The model diverges from a standard Leslie transition matrix in the adult stage. Within this stage, Heppell et al. (2002a) account for the variable breeding remigration intervals of adult females. Surviving adults are cycled through breeding and non-breeding years using remigration intervals calculated from tagged nesting females (Richardson 1978).

I used survival rates and stage durations estimated here for the benthic stage. I used the pelagic stage duration estimated in Chapter 3 with the new estimation of the duration of the benthic stage to approximate a total age-to-maturation. Heppell et al. (2002b) estimate 0.37 for pelagic stage annual survival rate in Kemp's ridleys. As this stage is approximately one year in duration for Kemp's (Chapter 4), I used this value in the fecundity function for loggerheads to estimate first year survival, not including nest and crawl survival. Heppell et al. (2002a) used fecundity data from TEWG (1998) and Frazer (1983). I used the same values in the current model: nests per breeding female = 4.1; eggs per nest = 115; survival from nest to water = 0.6747 (Frazer 1983, TEWG 1998). NMFS-SEFSC (2001) estimated sex ratios for the subpopulations of 0.35 for the northern subpopulation (NSP) and 0.80 for the south Florida subpopulation (SFSP) and I incorporated these values into the fecundity function. Models were run using the software program Mathcad™ (Math Soft, Inc.). To simulate population trends prior to 1990, I assumed the northern subpopulation was declining at 3% per year. All data including an initial λ=0.97 were input into the model to solve for pelagic stage annual survival rates after age one (Frazer 1983, Crouse et al. 1987).

To investigate the impact of the longer pelagic juvenile stage duration on proposed management scenarios (NMFS-SEFSC 2001, Anonymous 2001), I perturbed mortality rates similar to Heppell et al (2002a) and NMFS-SEFSC (2001). To simulate the impacts of increased survival of benthic juveniles under current TED regulations, I reduced mortality by 30%. To simulate the proposed expanded TED regulations incorporating larger-sized openings, I reduced mortality by 30% for all benthic juveniles, sub-adults and adults. I then looked at the same two TED regulation scenarios under increased and decreased mortality in the pelagic juvenile stage.

RESULTS

Benthic stage duration

The von Bertalanffy growth interval equation fit the growth interval data well ($r=0.99$, $P<0.001$), and resulted in an estimate of asymptotic length of 100.04 ± 7.01 S.E. cm SCL and an intrinsic rate of growth of 0.097 ± 0.02 S.E. For specimens where settlement could be detected, sizes-at-death and post-settlement ages were plotted along with the estimated von Bertalanffy curve to visually assess the fit of the curve (Fig. 5.1). Size specific growth rates declined with increasing size (Table 5.1). Starting with a settlement size (L_0) of 49 cm SCL (Chapter 3), the curve estimated 17 years to 90 cm SCL which is the average size at first reproduction for female loggerheads from the southeast U.S. (NMFS-SEFSC 2001). In Chapter 3, using a linear regression technique, the average duration of the pelagic stage was estimated at 14 ± 3.2 S.D. yrs. (N=84). Combining this value with the current estimate of the benthic immature stage gave an average age at reproductive maturity of 30.8 ± 3.2 S.D. years. The von Bertalanffy curve was used to

Table 5.1. Size-specific growth rates estimated from the outermost LAG diameters. Growth rates are given as mean ± standard deviation.

Size Category	N	Growth Rate (cm/yr.)
40-49 cm SCL	21	4.79 ± 0.37
50-59 cm SCL	26	3.90 ± 0.31
60-69 cm SCL	11	3.24 ± 0.24
70-79 cm SCL	8	2.15 ± 0.37

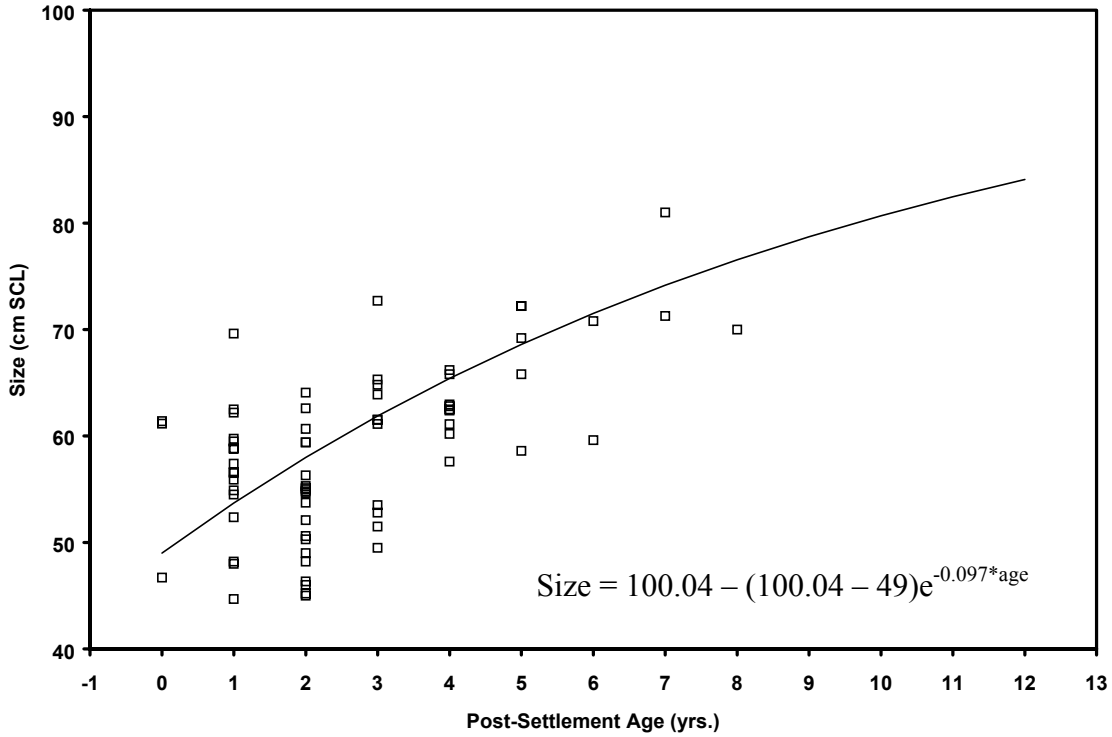


Figure 5.1. Size as a function of post-settlement age in loggerhead sea turtles. Line represents the von Bertalanffy growth curve created from growth interval data from animals up to 89 cm SCL (N=77). Open squares are post-settlement ages at death (N=70) to demonstrate the fit of the curve created by growth interval data to actual post-settlement age estimates.

estimate the time from size-at-settlement to 90 cm SCL for the specimens for which pelagic stage duration was estimated in Chapter 3 and resulted in a range of ages-at-maturation of 24.3 to 38.8 years.

Adult longevity

The relationship between average time since maturation and size was significant for males (P=0.02) but not for females (P=0.12) (Fig. 5.2). There are no published values for size-at-reproductive maturity for males in this population, but from the intercept of the regression, this value is estimated at 94.2 cm SCL (SE = 2.3). The oldest times since maturation in the samples were 26 years for males and 38 years for females. The mean time since maturation of the males was 16.0 years while for females it was 13.3 years.

Survival rates

The catch-curve created from the 1987 strandings data showed a marked change in the slope at 10 years after settlement to the benthos, which corresponded to about 80 cm SCL (Fig. 5.3). This timing corresponded with decreased growth rates indicative of the animals approaching maturation. Survivorship in sub-adult stages is usually higher in turtles (Heppell 1998). Thus, two different survival rates were estimated from the catch-curve, one for benthic juveniles of 0.7871 and one for sub-adults of 0.8821. In the most recent update to the loggerhead model, NMFS-SEFSC (2001) estimated an adult annual survival rate of 0.812 but concede that this value is likely an underestimate as emigration was not taken into account. Survival rates of adults should not vary greatly from sub-adults and in many turtle populations they are higher (Heppell 1998). Therefore I used the same value calculated for sub-adults as an estimate of the adult survival rate. Together with the assumption of a

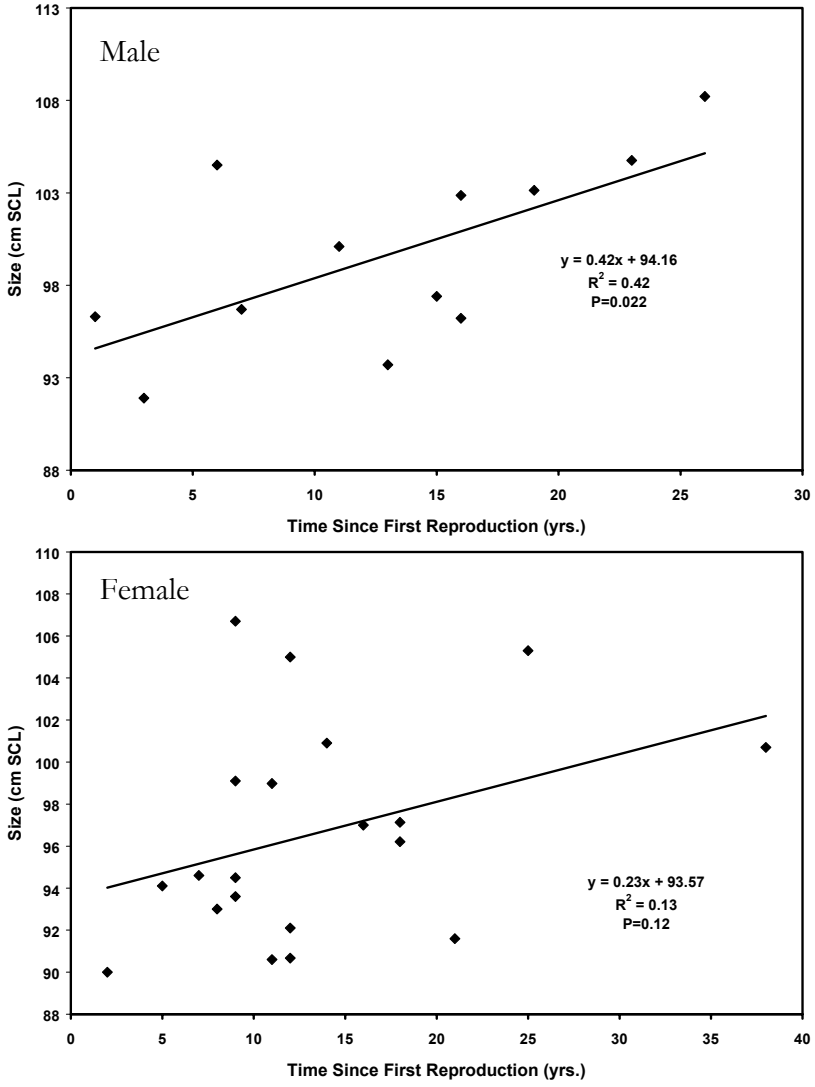


Figure 5.2. Size as a function of post-reproductive age in adult loggerheads. Solid lines are least-squares linear regressions.

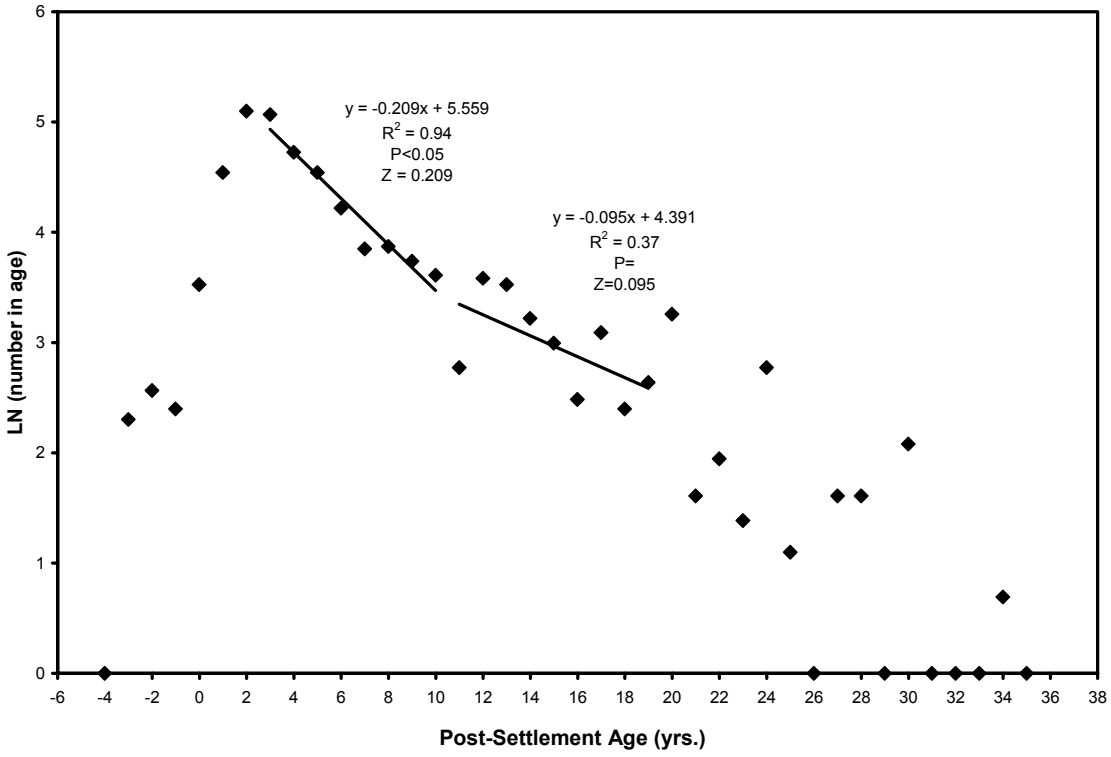


Figure 5.3. Catch-curve of 1987 loggerhead strandings data. Post-settlement ages were estimated from the SCL of the stranded turtles using the von Bertalanffy curve. Lines are linear regressions through portions of the data, from age 2 to 10 and from age 11 to 19.

declining population prior to 1990, these survival rates and stage durations resulted in a pelagic juvenile survival rate of 0.81 (Table 5.2).

Model results

I maintained the same five-stage structure of the Heppell et al. (2002a) but I termed the large benthic juvenile stage of Heppell et al. (2002a) as the sub-adult stage. Elasticity analysis of the model indicates that the pelagic juvenile stage has the highest elasticity, followed by the benthic juveniles for both of the female sex ratios (Fig. 5.4). Juvenile stage elasticities were proportional to the stage durations used in the model (Table 5.2). All else being equal, decreasing benthic juvenile survival by 30% did not result in recovering population growth rates for the northern subpopulation (NSP) but it was enough for the south Florida subpopulation (SFSP, Fig. 5.5). Decreasing mortality in benthic juveniles, sub-adults and adults through expanded TED regulations achieved stable or recovering population growth for both subpopulations (Fig.5.5).

When the same two scenarios were considered along with perturbations in pelagic mortality rates, decreasing pelagic juvenile annual mortality by 10% resulted in almost stable population growth rate for the NSP and recovering growth rates for the SFSP under the current TED regulations (Fig. 5.6). Under expanded TEDs, both subpopulations were increasing (Fig. 5.6). If, however, pelagic juvenile mortality rates are increased, the NSP declines under both TED scenarios. The SFSP is stable under current TEDs and increases under expanded TEDs (Fig. 5.6)

The model most likely to approximate current survival rates was current TED with a 10% increase in mortality for pelagic juveniles due to increases in the number of hooks being fished by the international longlining fleet in the Northern Atlantic Ocean (NMFS-

Table 5.2. Summary of parameters used in the current model. Survival rates from the original loggerhead matrix population model (Crouse et al. 1987) are included for comparison.

Stage	Size (cm SCL)	Duration	Survival Rate	Previous Survival Rates*
First Year	-	1 yr.	0.3700•	-
Pelagic Juvenile	<49	14 yrs.	0.8100	0.7857
Benthic Juvenile	49 – 80	10 yrs.	0.7871	0.6758
Sub-Adult	80 – 90	7 yrs.	0.8821	0.7425
Adult	>90	undefined	0.8821	0.8091

*From Frazer (1983) and Crouse et al. (1987)

•From Heppell et al. (2002b)

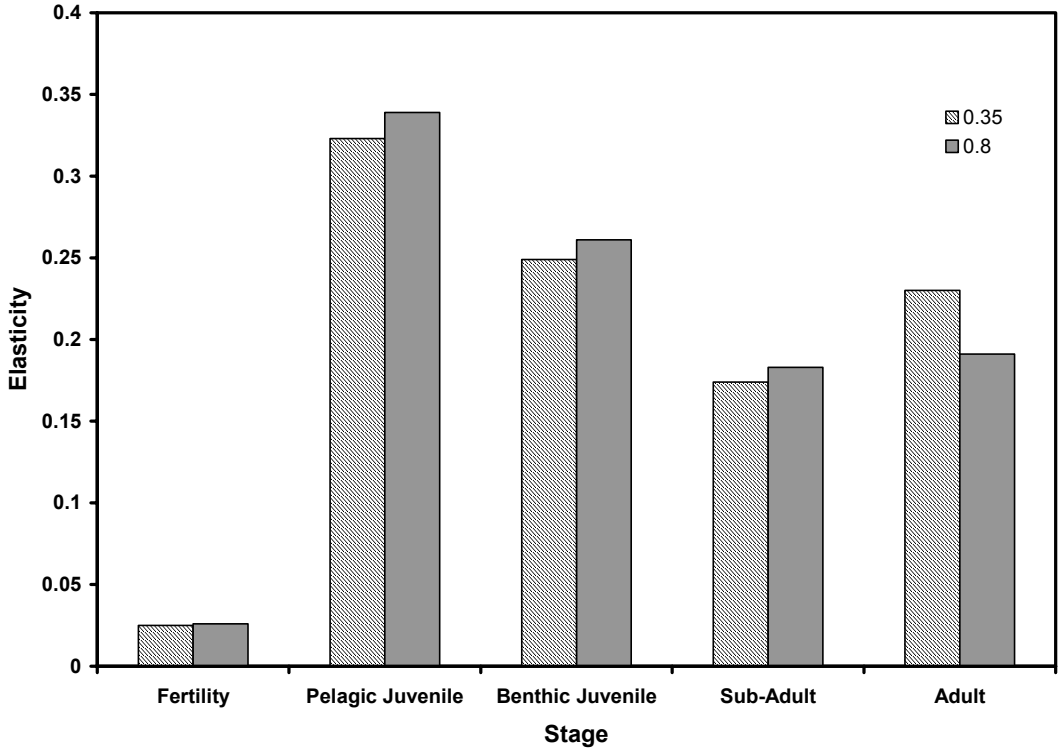


Figure 5.4. Stage elasticities from initial model with no perturbations in mortality rates, $\lambda = 0.97$ (representative of 1987). Hatched bars represent the northern subpopulation (35% female offspring), gray bars represent the south Florida subpopulation (80% female offspring).

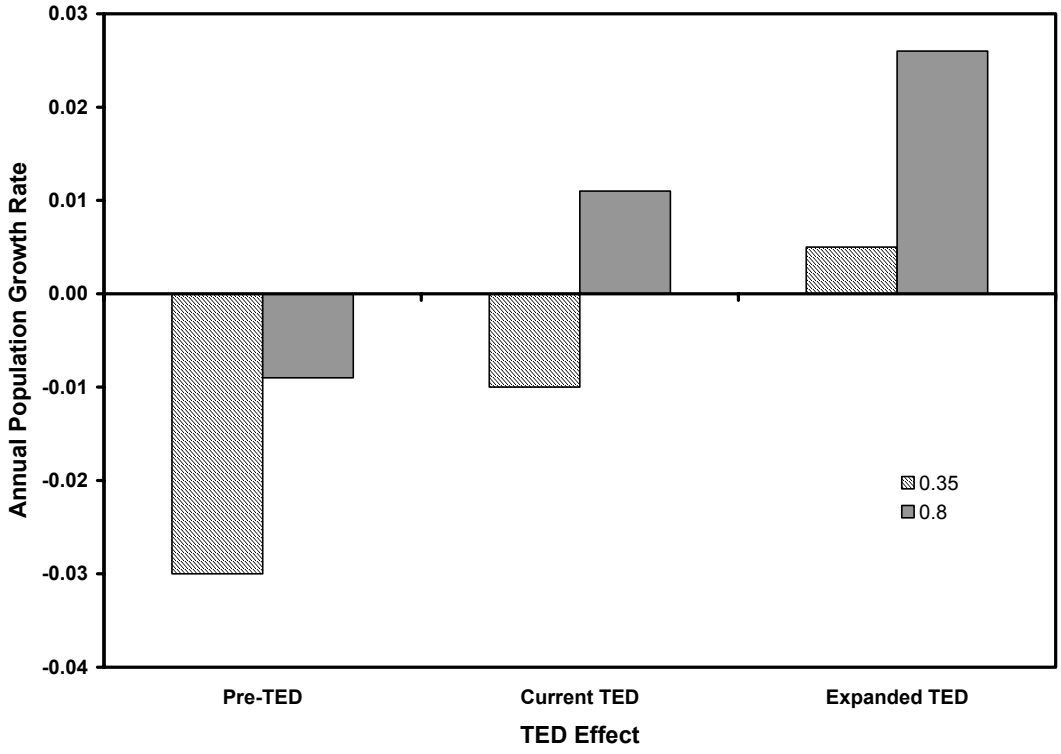
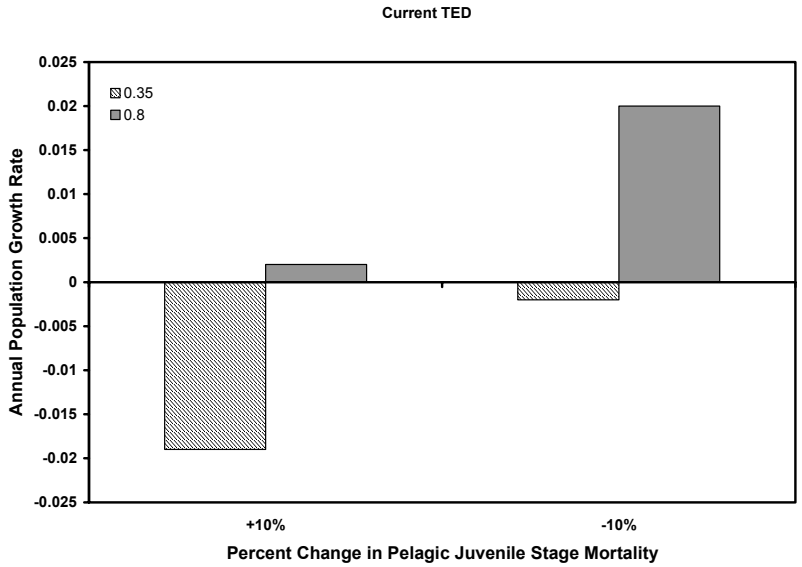


Figure 5.5. Resulting population growth rates when benthic juvenile mortality was decreased by 10% (Current TED) and when benthic juvenile, sub-adult and adult mortalities were reduced by 10%. The pre-TED $\lambda = 0.97$ for the northern subpopulation. Increasing the female offspring sex ratio to 0.80 resulted in a pre-TED $\lambda = 0.99$.



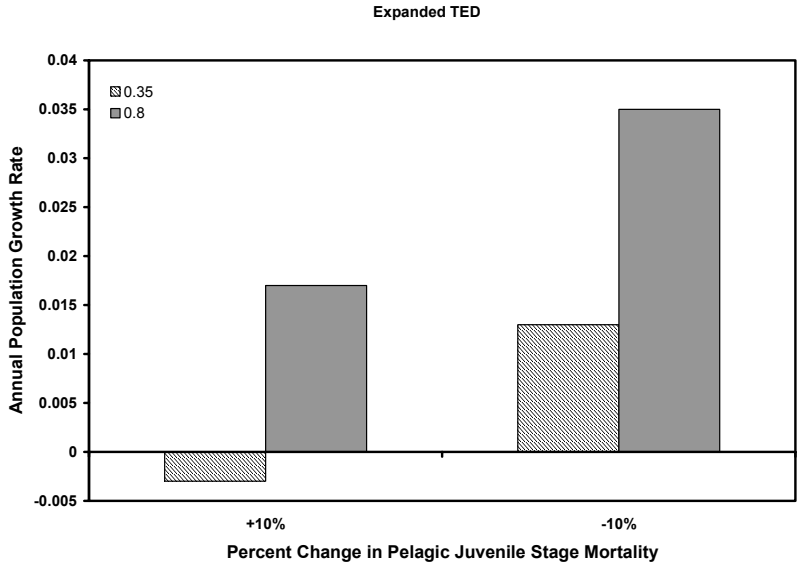


Figure 5.6. Resulting population growth rates when pelagic juvenile stage annual mortality rates are increased and decreased by 10%. Hatched bars represent the northern subpopulation (35% female offspring), gray bars represent the south Florida subpopulation (80% female offspring).

SEFSC 2001). This model was projected forward 100 years for both the NSP and the SFSP and the number of nesting females was plotted against time. The simulations were initialized with populations at stable age distribution under the 1987 mortality rate estimations and with 2000 breeding females (Fig. 5.7). These projections indicated how waves may be expected to pass through the adult population in response to perturbations in juvenile mortality rates. Based on numbers of nesting females only, populations appeared to increase and decrease for periods of time equivalent to decades.

Discussion

The longer pelagic juvenile stage duration for loggerheads documented in Chapter 3 and used in the model here changed the pattern of elasticities from what previous models have shown (Crowder et al. 1994, NMFS-SEFSC 2001, Heppell et al. 2002a). Proportional changes in mortality rates in the pelagic stage are predicted to have a greater impact on population growth rates than any of the other stages. However, based on stable age distributions, the majority of the loggerhead population at any one time is in this stage (approximately 90%). Thus, it is not likely that the anthropogenic or 'fishing' portion of the mortality is as great as for benthic, sub-adult and adult stages. In other words, we may not be able to affect the same proportional change in survival rates in pelagic stage juveniles as in benthic stage juveniles through management efforts. Even so, the results demonstrated here support the findings of NMFS-SEFSC (2001) that recovery of the northern subpopulation depends on decreasing mortality in all stages, and that small changes in pelagic stage survival rates can have large effects on λ.

Current trends in nesting females estimated from nest numbers are likely not indicative of population growth rates. When trends in the number of nesting females were

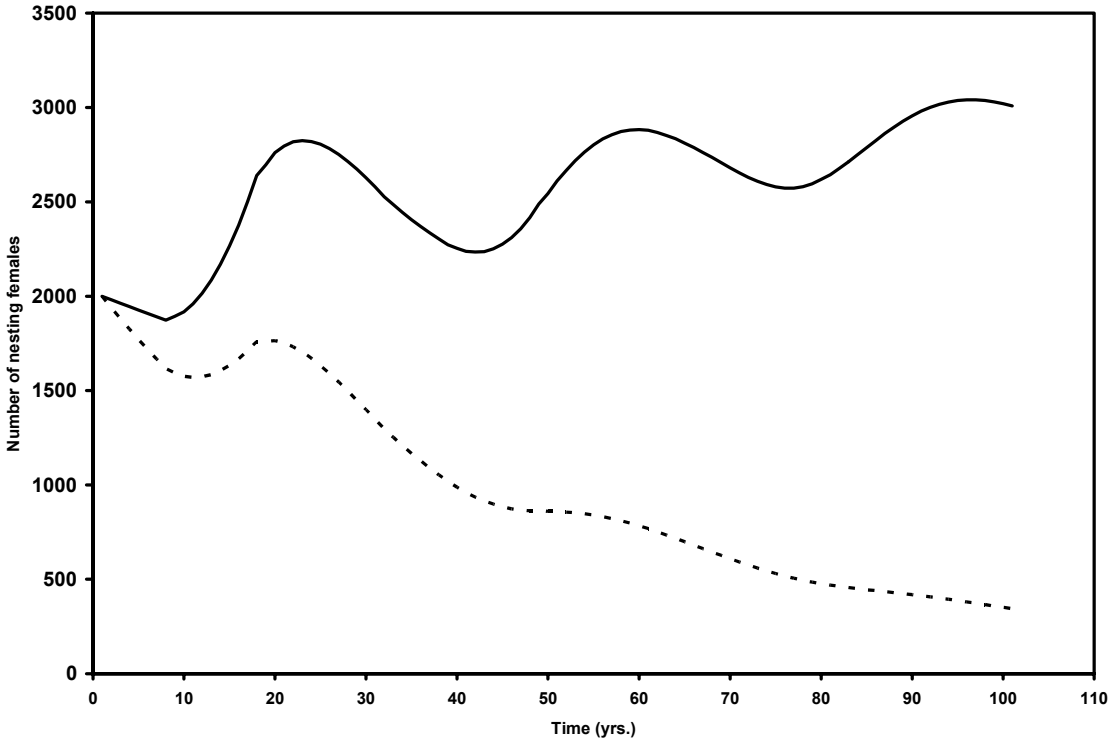


Figure 5.7. Population projections for models with benthic juvenile mortality reduced by 30% and pelagic stage mortality increased by 10%. Differences between the lines are the proportion of female offspring used in the fecundity function, solid line was 80% ($\lambda = 1.0$), dashed line was 35% ($\lambda = 0.98$).

modeled it was obvious how perturbations in the stable age distribution can cause waves to pass through the adult stage (see also Crowder et al. 1994). South Florida subpopulation appears to be increasing in size at a rate of about 3.6 % per year over the last decade (TEWG 2000). But from the population projections, increases of this magnitude are possible as a result of a sudden increase in survival of benthic juveniles, as brought about by implementation of TED regulations, though the actual population growth rate is approximately 0%. With constant fishing pressures, projected population trends indicated that the increasing trends in numbers of nesting females may begin to decline dramatically in the next 20 years or so, followed by increases and decreases on decadal scales until a stable age distribution is reached.

Because of the impacts of these perturbations in stable age structure and the high annual variability in nest numbers, management decisions based solely on nesting beach trends may be risky. This model is deterministic and there are numerous assumptions involved, hence the population projections can only be viewed qualitatively. However, the south Florida subpopulation may not be recovering at the rate that current nest numbers suggest. For this reason, efforts to assess the trends in other in-water stages are critical. Under the current model, it took 17 years for declines in pelagic stage survival to impact nesting beach trends. If juvenile recruitment out of the pelagia and into the benthos were monitored for example, by monitoring the smaller benthic size classes, decreases numbers within that stage would be detected early and management measures could be taken before the effects are seen on the nesting beaches.

Results of the model indicated that under the assumptions of current TED regulations and potential 10% increases in mortality due to increased longline fishing efforts

over the past 10 years (NMFS-SEFSC 2001), the South Florida subpopulation was approximately stable. Expanded TED regulations were enough to shift this subpopulation to recovering. The lower fecundity, in terms of female offspring produced, and the lower initial population growth rates of the Northern subpopulation make it more difficult to recover. As indicated by the model results, expanded TED regulations and pelagic mortality rates equivalent to 1989 would result in recovering the northern subpopulation. However, the number of hooks being fished in the North Atlantic has increased by about 50% over the last 10 years or so (NMFS-SEFSC 2001), which most likely means fishing mortality of pelagic stage loggerheads has also increased. Combinations of increased mortality and expanded TED regulations negate the benefits of the TEDs and result in stable or slightly declining populations. Thus it is imperative that mitigation measures that are currently underway to reduce mortality in the longline fishery continue for the U.S. fleet and be expanded to the international fleet (Anonymous 2001).

Heppell (1998) demonstrates that in most species of turtles, the adult stage has the highest elasticity. The primary exceptions to this are the southeast U.S. and Australian loggerhead sea turtle populations. Here it is the sub-adult (U.S) and juvenile (Australia) stages that have the highest survival elasticities. Elasticity results of the model presented here indicated that juveniles have higher elasticities than sub-adult and adult stages.

Annual survival rates estimated from tag returns on nesting beaches result in values between 0.79 and 0.83 (Frazer 1983, NMFS-SEFSC 2001) and models that have incorporated them found low elasticities for the adult stage (Crowder et al. 1994, Heppell et al. 2002a). NMFS-SEFSC (2001) noted that survival rates estimated by these means are likely low because they assume strict nest beach fidelity and do not account for emigration.

In a more statistically rigorous evaluation of recapture data for both adult males and females, Chaloupka and Limpus (2002) estimate an adult annual survival rate of 0.875 for loggerheads in Australia. The adult survival rate used here is consistent with that of Chaloupka and Limpus (2002) at 0.8821 and when a 30% reduction in mortality is applied, the value increases to 0.9175.

In order for a life history that incorporates extreme ages-to-maturity to work, large juvenile, sub-adult and adult survival rates must be very high (Stearns 1992). For fish and reptiles, there is a consistent relationship between age at first reproduction (α) and adult instantaneous mortality rates (Z) (Charnov et al. 2001). Specifically, $\alpha * Z \cong 2$, so, for age at first reproduction of 31 years, Z should be about 0.0645 which relates to an annual survival rate of 0.938.

The elasticity of the adult stage is proportional to the adult annual survival rate because elasticities scale with stage durations and higher survival rates equate to a longer adult stage. The average longevity of the adult stage was equivalent to or longer than that of the juvenile and sub-adult stages and the maximum longevity values suggested that the adult stage may be longer than juvenile and sub-adult stages combined. The potential management implications of this are clear. I used equation (15) from Heppell et al. (2000), to examine the difference in elasticities between an adult survival rates. The equation is

$$e_{\bar{F}} = \frac{\bar{P} - \lambda}{(\alpha - 1)\ \bar{P} - \alpha\lambda}$$

where $e_{\bar{F}}$ is the fertility elasticity, $\bar{P}$ is the age-invariant adult survival rate, and α is age to sexual maturation. Adult survival rates of 0.8821 and 0.938 were applied, λ and α were held constant at 0.97 and 31 respectively. At an adult survival rate of 0.8821, fecundity, juvenile and adult survival elasticities were 0.0243, 0.731, and 0.245 respectively. When adult

survival rate was increased to 0.938, the survival elasticities changed to 0.0165, 0.497, and 0.503. With increased adult survival rates, the elasticity pattern of the stages changed, with adults having the highest elasticity. It is possible that we do not have enough data on adults to accurately estimate annual survival rates. If we are underestimating them, then the elasticity patterns suggested by the models may not be accurate and more emphasis should be placed on protecting the adults as a means of increasing population growth rates. Fortunately, conservation measures aimed at protecting the in-water benthic stages, such as increasing the openings on TEDs will also serve to protect the adult stage.

The intrinsic rate of increase estimated here for the von Bertalanffy growth curve describing benthic juvenile growth was higher than most other studies have found (0.031 to 0.064; Henwood 1987, Schmidd 1998, NMFS-SEFSC 2001, Braun-McNeill et al. in prep) and close to the value of 0.115 estimated by Frazer (1987). Braun-McNeill et al. (in prep) estimate annual growth rates of 1.81 ± 1.15 cm/yr for 50-59 cm SCL loggerheads from North Carolina, 2.16 ± 1.61 cm/yr. for turtles 60-69 cm SCL and 2.41 ± 0.51 cm/yr for 70-79 cm SCL turtles. The growth rates presented here fell within the ranges reported by Braun-McNeill et al. (in prep) for the 60-79 cm SCL size class ranges (Table 5.1). It is unclear why the growth rates estimated for the 50-59 cm SCL range are lower in the Braun-McNeill (in prep), however, the values I described were consistent with expectations of declining growth rates with age.

Our understanding of loggerhead vital rates has increased substantially over the last decade. The current information for subpopulation structuring indicates that population modeling efforts need to become more spatially and temporally explicit, incorporating

interactions between the subpopulations via male migration. Efforts to collect spatially explicit information on vital rates and population trends need to continue.

CHAPTER 6

CONCLUSIONS AND THE IMPLICATIONS OF ONTOGENETIC HABITAT SHIFTS FOR THE POPULATION DYNAMICS OF LOGGERHEAD (*CARETTA CARETTA*) AND KEMP'S RIDLEY (*LEPIDOCHELYS KEMPI*) SEA TURTLES

I have investigated the application of skeletal growth marks to age estimation and growth rate analysis for loggerhead and Kemp's ridley sea turtles and the application of this technique alone and in combination with stable isotope analyses to identify ontogenetic shifts. The results highlighted the use of skeletochronology as a valuable tool for the rapid assessment of growth rates in species where similar data can only be painstakingly gathered over long time periods. When used in combination with stable isotope ratio analysis, diet shifts that corresponded to ontogenetic habitat shifts were detected.

For loggerheads (*Caretta caretta*), the identification of a settlement line that marked the shift from pelagic to benthic habitats allowed for investigations into the duration of the pelagic stage. Previous estimates for the duration of this stage are 6.5 to 7.0 years to 49 cm SCL from length frequency and skeletochronology (Bjorndal et al. 2000, in review). Results presented here indicate that the stage may be twice as long and may represent the major portion of the juvenile stage in loggerheads. This had the result of changing the elasticity pattern of the stages from what previous loggerhead population models have reported (Crowder et al. 1994, Heppell et al. 2002a) and implying that conservation of the pelagic stage is important to maintaining this population of loggerheads. A re-analysis of the existing population model with the new parameters highlighted the need for expanded TED regulations that protect all benthic juveniles, sub-adults and adults in combination with maintaining pelagic stage mortality rates equivalent to what they were before 1990.

For the Kemp's ridley (*Lepidochelys kempi*), the results presented here for growth rates and size at maturation are consistent with what has been reported in the literature (Caillouet et al. 1995, Schmid and Witzell 1997, Schmid 1998, TEWG 2000). I found additional evidence to support the suggestion by Chaloupka and Zug (1997) of polyphasic growth in the juvenile stage. The inflection point that occurs at the switch in growth compartments may be associated with a previously unrecognized ontogenetic shift. It is unclear if the shift is occurring from a habitat switch, a change in resource use or a physiological shift.

Potential trade-offs between growth and mortality

From captive studies, loggerheads appear to be capable of growing at rates similar to Kemp's (Swingle et al 1993, Marquez 1994, Ben Higgins, pers. comm.). In the wild, however, their growth rates were different. Loggerheads from the southeast U.S. spend an average of 14 years in the pelagic experiencing low growth rates (2.90 cm/yr), followed by a shift to benthic habitats at an average size of 48.5 to 51.1 cm SCL (Chapter 3). Juvenile Kemp's ridleys that use coastal Atlantic habitats appear to spend only one year in the pelagia, then use coastal benthic habitats starting at about 24 cm SCL and experience about 5.00 cm/yr growth rates (Chapter 4).

Small juvenile loggerheads from the southeast U.S. are apparently not growing at their physiological maximum. Werner and Gilliam (1984) and Werner (1988) suggest that when an animal is vulnerable to size-specific predation in an optimal foraging habitat (habitat 2), it will use a sub-optimal habitat (habitat 1) where it is not vulnerable to predation as a means of minimizing mortality but with the consequence of reducing individual growth rates. Once a size-threshold is reached where the mortality risks of habitat 2 are reduced, the animal undergoes an ontogenetic habitat shift. Let us assume that the annual survival

rate for Kemp's is representative of what the rate would be for similar-sized loggerheads in the benthic habitat. According to the hypothesis of Werner and Gilliam (1984) and Werner (1988), small juvenile Kemp's should be subjected to higher mortality rates in the benthos than are juvenile loggerheads in the pelagic.

For Kemp's, the annual survival rate for small benthic juveniles (ages two to six years) is estimated at 0.61 (pre-1990; Heppell et al. 2002b). For loggerheads, the pelagic juvenile annual survival rate was estimated at 0.81 (pre-1990; Chapter 5). These numbers must be viewed with caution as survival rates in sea turtles, especially during the pelagic stage, are not well understood and there is likely a large amount of spatial, temporal, and size-specific variability inherent in them. However, these numbers give an indication that the pelagic habitat provides juvenile loggerheads with decreased mortality but at the cost of decreased growth rates.

Heppell et al. (2002b) detect a change in annual survival rates starting at about age six years in Kemp's based on a catch-curve analysis. They fit the model to estimate the annual survival rates of this age/size-class with the result of 0.84 to 0.85 annual survival prior to 1990. A seven-year-old Kemp's is approximately 42 to 50 cm SCL (Table 4.2, Chapter 4) which corresponds to the size at which loggerheads switch from pelagic to benthic habitats (48.1 to 51.1 cm SCL). The annual survival rate estimated for newly recruited benthic loggerheads was 0.79 (pre-1990). It is possible that this is a critical size-threshold for sea turtles after which mortality from natural sources may be minimized.

The life history of juvenile loggerhead sea turtles supports the hypothesis of Werner and Gilliam (1984). They appear to be trading-off higher growth rates and shorter age to maturation for increased juvenile survival. Kemp's ridleys, on the other hand, appear to be

taking the opposite tact and by spending a greater portion of the juvenile stage in the benthos encounter higher mortality rates but greatly decreased time to maturation.

Implications of ontogenetic shifts for population dynamics

The large-scale habitat segregation of the juvenile stages of loggerheads has implications for their population dynamics. The degree of overlap between ontogenetic stages influences population stability, for example, by influencing how much the density of one size class effects the density of another size class through intra-specific competition (Gilliam and Fraser 1988). By utilizing pelagic habitats, small juvenile loggerheads do not compete with large juveniles or adults for resources. There is a great deal of variability in the timing of the transition between habitats, indicating that animals of similar ages and/or sizes are subjected to different growth and mortality rates, leading to variations in the population dynamics.

The habitat shift in Kemp's implied by the variance in growth rates may be a similar mechanism whereby smaller Kemp's are using a different habitat or resource than are large juvenile and adult Kemp's. The two major developmental habitats for juvenile Kemp's, Atlantic Coast or Gulf of Mexico (Collard 1990, Collard and Ogren 1990), and the different growth rate potentials presented by each may be an additional means of decreasing the potential of intra-specific competition but similarly complicating the population dynamics.

Future Research

The techniques presented here need to be applied to individuals of these same populations of loggerheads and Kemp's for additional information on habitat use and habitat specific growth rates to broaden our understanding of the variability inherent in these parameters and the implications for population dynamics. In addition, the techniques

can be applied to additional populations and species of sea turtles for similar insights into their life histories. Though the use of rapprochement of the LAG's for estimation of post-reproductive longevity needs to be validated, this can provide extremely valuable information on population stability, adult survival, and reproductive intervals for both males and females.

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BIOGRAPHY

Melissa L. Snover

DATE AND PLACE OF BIRTH: 9 June, 1964, Endicott, NY, USA

EDUCATION

M.Sc., Hood College, Frederick, Maryland, 1994.

Major: Environmental Biology

Thesis Title: The application of fractal geometry to the analysis of *Mytilus edulis* spatial distribution in a soft-bottom system.

Description: I studied an intertidal population of mussels on the coast of Maine. The fractal dimension of the outline of the mussels against the substrate was measured and related to the spatial distribution of the mussels. I took aerial-view photographs of the mussels and analyzed them for fractal dimension, percent cover, and degree of spatial aggregation. The results showed a strong parabolic relationship between the percent cover of the mussels and the fractal dimension (r^2=0.94). I found a negative linear relationship between fractal dimension and the degree of spatial aggregation (r^2=0.84).

B.A., State University of New York at Potsdam, Potsdam, New York, 1986.

Majors: Biology
Mathematics

Additional Emphasis: Chemistry

HONORS AND AWARDS

2000 Runner-up, Best Student Paper, 20th Annual Symposium on Sea Turtle Conservation and Biology

1999 Runner-up, Best Student Paper, 18th Annual Symposium on Sea Turtle Conservation and Biology

PUBLICATIONS

Snover, M.L. and J.A. Commito. 1998. The fractal geometry of *Mytilus edulis* L. spatial distribution in a soft-bottom system. Journal of Experimental Marine Biology and Ecology. 223, 53-64.

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